

Classification of Microorganisms - Taxonomy

I. By carbon and energy source.

Carbon source + energy source + nutrients → new cells + heat + other products

	Carbon	
Energy	chemoheterotrophs	Chemoautotrophs
	Photoheterotrophs	Photoautotrophs

II. By cell structure

Prokaryotic - eubacteria, archebacteria

Eukaryotic - fungi, yeasts, algae, protzoa, higher plants and animals

A. Prokaryotic

Single DNA molecule

Only membrane is the cell membrane

Peptidoglycan cell wall

Reproduction by binary fission

Motility by means of flagella in many

Small size, great diversity, high growth rates, ubiquitous

B. Eukaryotic

Larger size

Several DNA molecules organized into chromosomes and surrounded by nucleus

Presence of organelles

C. Viruses - obligate intracellular parasites

III. By genetic sequencing

Evolutionary relationships between groups of organisms

Comparative sequencing of 16S or 18S ribosomal RNA

Three main branches - Eucaryotes, Eubacteria, Archaea

IV. By cell shape - morphology

Spheres - cocci

Rods - bacilli

Spirals - spirilla

V. By gram stain

Reaction with stain is due to differences in cell wall structure and chemistry

VI. By how they grow

Floc forming - grow in clumps

Dispersed - individually

Biofilm - attached to surface

VII. By O₂ requirements as electron acceptor

Aerobic

Anoxic

Anaerobic

Facultative

Microaerophilic

VIII. Other Classification Methods

By whether they cause disease - pathogens

By optimal temperature for growth - thermophilic, mesophilic, psychophilic

Ability to form endospores - spore forming

Acid tolerance - acidophilic

Salt tolerance - halophilic

Dry conditions - xerophilic

Nutrient Requirements - oligotrophic, eutrophic

Cell Chemistry

80% water - main constituent of cell protoplasm, required for transport processes

20% dry matter:

90% organic compounds

10% inorganic compounds

Major elements - C, O, H, N, S, P

Empirical formula $C_5H_7O_2N$

Trace elements - see chart

Hierarchy of cell organization:

precursors → monomers → polymers → cell components → cell

4 Types of macromolecules (polymers)

Informational (proteins, nucleic acids) Non-informational (lipids, polysaccharides)

Polysaccharides:

Cell walls, storage polymers, structural backbone of nucleic acids

Composed of carbohydrates $[CH_2O]_n$ connected by covalent (glycosidic) bonds.

Can also combine with proteins and lipids

Lipids and Fats:

Cell membrane

Composed of fatty acids (has hydrophobic and hydrophilic groups)

Nucleic Acids: DNA RNA

Composed of Nucleotides

Purine bases - adenine, guanine

Pyrimidine bases - thymine, cytosine, uracil

Information of nucleic acids is determined by the base sequences

Proteins

Enzymes, structural proteins

Composed of Amino Acids

Amino acids are assembled into a specific sequence

Enormous diversity

Parts of the Prokaryotic Microbial Cell

I. Cell membrane - phospholipid bilayer with inserted proteins - selectively permeable.

Energy Synthesis

Transport processes

Osmosis

Membrane transport proteins

Facilitated diffusion

Group translocation

Active transport

II. Cytoplasm - liquid contained in the cell - water dissolved nutrients, protein, RNA

III. Cell wall - rigid layer of polysaccharide - peptidoglycan - glycan strands crosslinked by peptide bonds - unique to prokaryotes

Stabilizes cell

Prevents lysis

Determines cell shape

Plays a role in cell division and motility

Permeable

Gram (-) thin layer of peptidoglycan plus an outer membrane

Gram (+) thick layer of peptidoglycan with teichoic acids

IV. Nuclear region

Single, double stranded DNA molecule

Naked, covalently closed

Highly folded with primary and secondary structure

V. Plasmids - small closed strand of DNA

Optional - not strictly necessary for growth

Encodes specialized functions - e.g. antibiotic resistance, pathway for degradation of a specific compound

Role in genetic exchange

Selective pressure to lose if not needed

VI. Ribosomes - site of protein synthesis

Composed of protein and RNA

VII. Flagella - long thin appendages

Made of protein

Rotates like a propeller to move organisms in medium

Chemotaxis - movement toward or away from a chemical

Phototaxis

Random walk

VIII. Fimbriae - thin appendages used in attachment

IX. Pili - thin appendages used in genetic exchange

Congugation - transfer of a plasmid

X. Capsules or slime layer - polysaccharide layer on outside of cell

Role in attachment

Storage polymer

XI. Storage granules - PHB, glycogen, phosphorus, sulfur

XII. Gas vesicles - controls cell buoyancy

XIII. Endospores - cells that resist hard times

Chemical Thermodynamics Review

- ❖ Most biological reactions are oxidation reduction reactions. A redox reaction is a coupled reaction that involves the transfer of electrons from one molecule to another.
 - Oxidation - the molecule or atom loses electrons.
 - Usually we can tell a compound is oxidized because it gains oxygen, loses hydrogen or gains a more positive charge.
 - The compound that is oxidized can be referred to as an electron donor or a reducing agent.
 - Reduction - the molecule or atom gains electrons.
 - Usually we can tell a compound is reduced because it loses oxygen, gains hydrogen or gains a more negative charge.
 - The compound that is reduced can be referred to as an electron acceptor or an oxidizing agent.
 - The two molecules involved are called a redox pair.
- ❖ Balancing redox reactions by the half reaction method (acidic or neutral conditions):
 - Step 1. Write the reaction as two half reactions, one oxidation and one reduction.
 - Step 2. Balance all compounds that are not O or H.
 - Step 3. Balance O by adding H₂O to the side deficient in O.
 - Step 4. Balance H by adding H⁺ to the side deficient in H.
 - Step 5. Balance charge by adding e⁻ to the more positive charge.
 - Step 6. Multiply each half reaction by some factor to have the same number of electrons transferred in each half reaction.
 - Step 7. Add the two half reactions together and clean up.
 - Step 8. Check that all elements and charge are in balance.
- ❖ **Free energy of a reaction** - when a chemical reaction takes place, a change in energy occurs. The amount of energy released can be expressed as:
 - H - enthalpy - total amount of energy released
 - G - Gibbs free energy - energy available to do useful work $\Delta G = \Delta H - T\Delta S$
 - If ΔG is negative, energy is released (the reaction is spontaneous) if ΔG is positive, energy is needed to drive the reaction (the reverse reaction is spontaneous).

❖ The change in free energy during a reaction can be computed from:

$$\Delta G = \Delta G^{\circ} + RT \ln \left[\frac{[C]^c [D]^d}{[A]^a [B]^b} \right] = \Delta G^{\circ} + RT \ln Q$$

where ΔG is the change in free energy under the given conditions (kJ), ΔG° is the standard change in free energy at pH 7 (kJ), R is the ideal gas constant, T is the absolute temperature.

⇒ **Free energy of formation** (G_f°) - the energy released or required to form one mole of molecules from its elements. These values are tabulated (see Appendix A in your textbook). By convention the G_f° of the elements (O_2 , N_2 , C) is zero. One can calculate the free energy for a reaction using a balanced chemical equation and the tabulated G_f° values as:

$$\Delta G^{\circ} = \sum G_f^{\circ} \text{ products} - \sum G_f^{\circ} \text{ reactants}$$

The difference in electrical potential between two redox pairs is expressed as the ΔE_o , and is directly proportional to ΔG° :

$$\Delta G^{\circ} = -nF\Delta E_o$$

where n is the number of electrons transferred and F is Faraday constant (96,630 J/V).

⇒ **Free energy and equilibrium** - at equilibrium the forward rate of reaction is equal to the reverse rate of reaction and:

$$0 = \Delta G^{\circ} + RT \ln \left[\frac{[C]^c [D]^d}{[A]^a [B]^b} \right] = \Delta G^{\circ} + RT \ln [K_{eq}]$$

$$K_{eq} = \exp \left(\frac{-\Delta G^{\circ}}{RT} \right)$$

⇒ **The Electron Tower**

Half reactions are arranged so the couples with the most negative reduction potentials are at the top of the table and the most positive reduction potentials are at the bottom. All half reactions are shown as reductions. This arrangement can be thought of as an electron tower. Compounds at the top of the tower are good electron donors and compounds at the top are good electron acceptors. The greater the difference in potential between two half reactions in the tower, the greater the amount of energy from the reaction.

⇒ **Activation Energy** - E_A - energy required to bring compounds to a reactive state. For example, energy may be required to break bonds in the molecules before they can react.

Measurement of Organic Carbon

Importance - organic substrates, degree of purification or treatment efficiency, biodegradability of substrate.

Trace measurements of organic carbon - measurement of individual species by chromatography, wet chemical techniques.

Bulk measurements - what we'll do in this class - lump all organic matter together:

TOC - total organic carbon - convert organic C to CO₂ and measure IR absorption

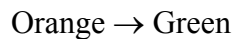
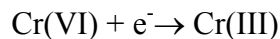
BOD - Biochemical oxygen demand - most widely used parameter in wastewater effluents. Historical significance. Operationally defined parameter - measures DO used by microorganisms under specified conditions over specific time period.

Put waste, seed, nutrients, DO and water in bottle. Measure initial DO (D₀), incubate fixed time (t) at 20 °C, measure DO again (D_t).

$$BOD_t = \frac{[(D_0 - D_t) - f(B_0 - B_t)]}{P}$$

Importance of P (mL wastewater/mL dilution water + wastewater) - need to “hit the window” i.e. provide enough wastewater to have a measurable DO depletion but not so much wastewater that all of the DO is gone. Since we don't know BOD_t in advance, we typically do multiple dilutions.

COD - chemical oxygen demand - measures the amount of organic matter that is chemically oxidized using a strong oxidant (potassium dichromate), at high temperature, and in the presence of a catalyst. Measure color change using a spectrophotometer resulting from dichromate reduction:



Convert to O₂ equivalents (COD) using a calibration curve with a compound (KHP) with known COD.

COD>BOD since both biodegradable and recalcitrant compounds are oxidized in COD test. BOD:COD ratio can be used as a measure of biodegradability.

ThOD - theoretical oxygen demand - redox equation complete oxidation of the organic compound (if chemical formula is known or can be estimated). g-O₂ required/g organic carbon

Enzymes

Activation Energy - E_A - energy required to bring compounds to a reactive state. For example, energy may be required to break bonds in the molecules before they can react.

Biological Catalysts - substance that lowers activation energy - substance is not changed in the process

Proteins twisted and folded into a specific shape - lock and key analogy.

Increases reaction rate $10^8 - 10^{20}$ times

Places strain on bonds to reduce E_A

⇒ Mathematical expression for enzyme kinetics

- $[E] + [S] \rightleftharpoons [ES] \Rightarrow [P]$
- where E = enzyme, S = substrate, ES = Enzyme substrate complex, P = product
- From a mass balance on E we get:

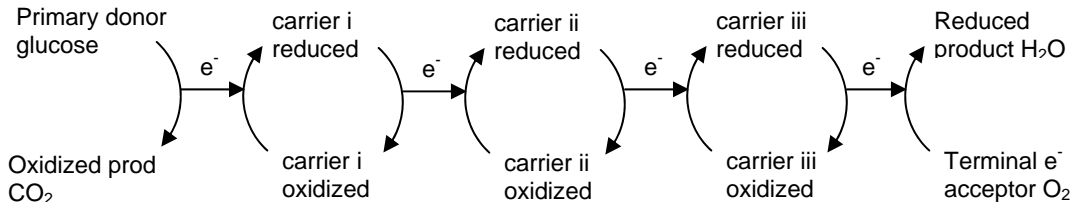
- $$\frac{dP}{dt} = V_o = \frac{V_{\max}[S]}{K_m + [S]}$$

- where: V_{\max} = maximum rate of P production, K_m = half saturation constant
- Linweaver – Burke plot. Plot $1/V_o$ vs. $1/S$ and determine K_m and V_{\max}
- Note that these equations were developed for simple enzyme reactions. Enzyme reactions can be much more complex. In a cell many different enzymes are working and many different processes are occurring.

Microbial Metabolism

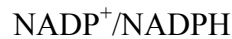
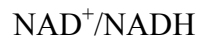
Electron carriers - series of oxidation reduction reactions

$\sum \Delta G = \Delta G$ for reaction between primary donor and terminal acceptor



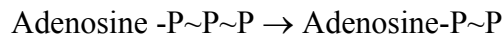
Kinds of carriers:

Freely diffusable -good electron donors



Membrane bound electron transport chain - flavoproteins, quinones, cytochromes

High energy phosphate carriers - The “energy coin” of the cell

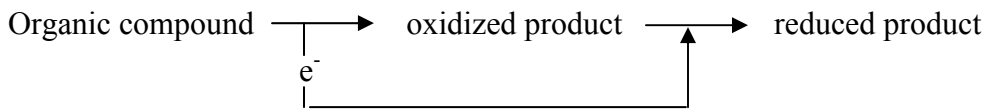


Two ways to make ATP

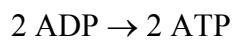
Substrate level phosphorylation - series of reactions with a substrate

Proton motive force - cell acts as a proton pump

Fermentation - only substrate level phosphorylation



Example: glucose \rightarrow 2 ethanol + 2 CO₂



Why do they do it?

No terminal electron acceptor around

Able to fill ecological niche

Products - yogurt, cheese, beer

Anaerobic treatment processes

Respiration

In the presence of O_2 , NO_3^- , SO_4^{2-} , Fe^{3+} or another terminal electron acceptor, the carbon source can be completely oxidized to CO_2 through:

Tricarboxylic acid cycle (TCA cycle)

Proton motive force

TCA cycle - complete oxidation of one mole of glucose to CO_2 and H_2O

1. Glycolysis: glucose \rightarrow 2 pyruvate + 2 ATP + 2 NADH
2. Each pyruvate is decarboxylated to Acetyl-CoA + 1 NADH + 1 CO_2
3. Acetyl group of acetyl-CoA combines with oxalacetate to form citrate
4. Citrate goes through a series of reactions - oxalacetate is regenerated + 2 CO_2 + 3 NADH + 1 FADH + 1 ATP

(key intermediates in the TCA cycle - citrate, α -ketoglutarate, succinate, oxalacetate)

Proton Motive force

Electrons from NADH are passed off to the electron transport chain used to pump H^+ out of the cell.

ATPase - membrane bound enzyme catalyzes the reaction $ADP \rightarrow ATP$

Microbial Metabolism Review

1. Cell couples energy from oxidation reduction reactions
2. A first approximation for the energy available for a reaction can be obtained from ΔG°
 - a) Using free energy of formation method
 - b) Using Faraday's law
3. The electron tower - a useful tool to visualize the energy available from the rxn between an electron donor/electron acceptor pair
4. The transfer of electrons from a primary donor to a terminal acceptor involves intermediates called electron carriers
 - a) Freely diffusible NAD⁺/NADH
 - b) Membrane bound electron transport chain
5. Cells need ATP to carry out biosynthesis and other cell functions
6. Cells make ATP two ways:
 - a) Substrate level phosphorylation - substrate goes through a biochemical pathway in which ATP is made
 - b) Proton motive force - H⁺ is pumped out of the cell to create a gradient - some of this potential energy is given up and ATP is formed through the action of ATPase.
7. Fermentation - a way for heterotrophs to make ATP in the absence of a terminal electron acceptor. Not much ATP is made. High energy waste products are produced.
8. Respiration - if a terminal electron acceptor is available organism can use both substrate level phosphorylation and proton motive force to generate ATP. Central metabolic cycle is TCA (citric acid) cycle.

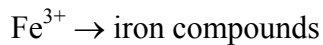
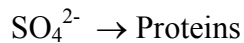
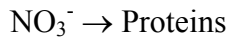
Alternate Modes of Energy Metabolism

If no O_2 is present, other oxidized compounds can serve as terminal electron acceptors for heterotrophic metabolism.

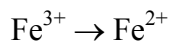
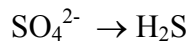
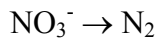
Anoxic Respiration - uses TCA cycle to oxidize organic carbon and reduce NAD^+ to $NADH$

Definitions

Assimilatory Reduction



Dissimilatory reduction



Denitrification

- Reaction is less energetically favorable than aerobic respiration
- Mostly carried out by facultative bacteria
- Reaction can also produce N_2O and NO
- Used in wastewater treatment, drinking water treatment, air pollution control
- Implications for soil fertility

Sulfate Reduction

- Organisms can use fermentation end products such as ethanol and pyruvate as electron donors
- First step in sulfate reduction requires ATP
- Alkalinity produced and metal sulfide production can be used to remediate acid mine drainage (AMD) sites - bioreactors or wetlands treatment.
- H_2S - highly odorous compound - sewer gas - reacts with Fe to produce black FeS

Iron Reduction

- Fe^{3+} is common in soil and rock
- Plays significant role in natural attenuation of hydrocarbons
- Fe^{2+} significant groundwater contaminant

Other Elements: Mn, Se, As

Organic electron acceptors

- Reductive dehalogenation - significant in clean up of sites contaminated with chlorinated hydrocarbons

Development of TEAP zones

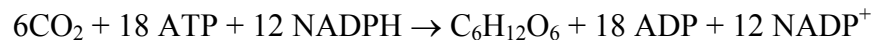
Terminal Electron Accepting Process

From Atlas and Bartha, 1998: Microorganisms within the community use the specific electron acceptor they are genetically capable of using that yields the maximal energy yield from the available substrate. Each electron acceptor is utilized at different redox potentials. This seemingly intelligent decision is in part due to metabolic regulation within a single population, and in part due to the inevitable outcome of competition between populations with diverse metabolic capabilities.

Chemolithotrophic Metabolism

- Energy from oxidation of inorganic compounds - H_2S , NH_3 , NO_2^- , Fe^{2+}
- Carbon from CO_2 - autotrophs
- Important role in cycling Fe, S, N in the environment
- Important role in environmental engineering - nitrification, H_2S oxidation

CO_2 Fixation



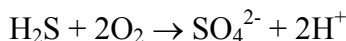
- Proceeds through a pathway called the Calvin cycle
- Requires a lot of energy in the form of ATP
- Requires a lot of reducing power in the form of NADPH

The problem for Chemolithotrophs - electrons can't run uphill without input of energy

$\text{NADP}^+/\text{NADPH}$	-0.32
$\text{SO}_4^{2-}/\text{H}_2\text{S}$	-0.22
$\frac{1}{2} \text{O}_2/\text{H}_2\text{O}$	+0.82

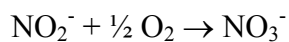
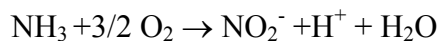
The solution - reverse electron transport

Sulfur Oxidizing Bacteria - importance in odor control through biofiltration, geochemical sulfur cycle, AMD generation:

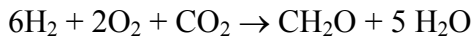


- Low energy yield
- Low growth rate
- Obligate aerobic bacteria
- Acidophiles

Nitrifying bacteria-importance in wastewater treatment, soil fertility, groundwater contamination:



Hydrogen bacteria- hydrogen is high enough on the electron tower, these organisms don't need reverse electron transport to generate reducing power. They can also use other electron acceptors, such as NO_3^- , ClO_4^- , SO_4^{2-}



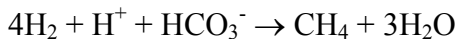
Fe oxidizing bacteria - important in AMD generation



Acetogenic bacteria - important role in anaerobic carbon cycle



Methanogens

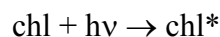


Fueling Reactions in Photosynthesis

Chlorophyll exists in 3 states:

Ground state chl

Excited state chl*



Oxidized state chl⁺

Photosystem I - Cyclic electron transport - used to make ATP

All occurs in the cell membrane with chl as both donor and acceptor

No electrons can be drawn off to produce reducing power

Three strategies for dealing with carbon in phototrophic organisms

1. Purple bacteria - photoheterotrophs - use organic carbon as carbon source, photosystem I as source of ATP. Large amount of reducing power not needed.
2. Green bacteria - anoxygenic photosynthesis uses reduced sulfur compounds and photosystem I to make NADPH - fixes inorganic C
3. Cyanobacteria, algae, green plants - oxygenic photosynthesis - 2 photosystems. Gets electrons by oxidizing H_2O to O_2 . Water is a very poor reducing agent but readily available. The use of two photosystems increases the potential enough to produce NADPH.

Measurement of Microbial Biomass

- ⇒ Cell mass – determine mass of cells and report in mg/L. Does not distinguish cells from inert material, does not distinguish live and dead cells.
 - Net mass – centrifuge cells and weigh pellet
 - Dry mass – dry cell suspension, pellet, or filtered suspension. Suspended solids (SS) weigh filter, filter suspension, dry to constant weight (100-105 C), and reweigh filter.
 - Volatile suspended solids (VSS) – take filter from dry weight measurement and volatilize organic fraction at 550 C. The ash left is inorganic. Determine VSS by difference.
- ⇒ Direct counts – visual count of cells under a microscope.
 - Doesn't distinguish live from dead cells
 - Can't use with soil or inert solids
 - Can't be used with very dilute suspensions
 - Small sample size – heterogeneity
 - Small, motile cells difficult to count
- ⇒ Turbidity (optical density) – measure of the light scattered by suspended material. Report as NTU, OD, Klett units. Can also use a coulter counter.
 - Not always proportional to other measures of growth
 - Does not distinguish live from dead cells
 - Quick and easy
- ⇒ Viable counts – culture cells in some growth medium and measure live or viable cells. A selective media can be used.
 - Plate counts – grow cell colonies on solid media. Nutrient solution to which agar has been added
 - Prepare dilutions. Dilute to the point where you have 30-300 colonies per plate. Each colony grows from a single cell.
 - Pour plates. Add sample to medium. Colonies grow imbedded in medium.
 - Spread plates. Colonies grow on the surface of the media.
 - Membrane filter – colonies grow on the filter.
 - Can also use test tubes instead of plates – slants.

- Most probable number (MPN) statistical method based on Poisson distribution.
 - Dilute sample until there is less than one cell per dilution level. Prepare multiple samples at each dilution level.
 - Score the samples as positive or negative based on:
 - Turbidity
 - Disappearance of a substrate
 - Ability to ferment a substrate.
 - Problems with viable counts
 - Usually 1-3 orders of magnitude less than direct counts
 - May need a long time for growth to occur
 - No one medium will support all organisms (may be an advantage)
 - Not all organisms will grow on plates or liquid culture
 - May need a certain population density before growth can occur.
- ⇒ Measurement of cell constituent
- ATP – easy to measure, all cells contain, turned over rapidly in dead cells.
 - Protein – all cells contain, but persistent.
 - Chlorophyll – selective for phototrophs .
 - Lipopolysaccharide – selective for gram negative bacteria.
 - Muramic acid – procaryotes
 - Chiten – fungi
 - Problems: concentrations of an indicator constituent may vary between species or with the same species at different growth phases. Compounds in dead cells also measured.
- ⇒ Measurement of cell activity.
- Substrate uptake
 - O₂ uptake.
 - CO₂ evolution
 - Radioactive tracer.
 - Problems – non biological transformations
- ⇒ Molecular Tools - see article by Lovely.
- Oligonucleotide probing - selects for specific sequence in target cells RNA
 - Fluorescence in situ hybridization (FISH) - can give information on special relationships of different organisms.
 - Polymerase Chain Reaction (PCR) - can probe for a specific strain, group of organisms with specific capability (e.g. methanogens), or larger groupings.

Genetics and Information Flow

Genetic material directs the process of protein synthesis (structural proteins and enzymes). All cell functions involve the actions of specific proteins.

Gene – “packet “ of information

DNA – deoxyribosenucleic acid – large macromolecule genes are encoded in sequences of bases on the molecule.

Chemistry of DNA and RNA – molecules have three parts:

- 1) Five carbon sugar (DNA – deoxyribose, RNA – ribose)
- 2) N containing base
 - a) Purine bases (2 rings) - adenine (A) and guanine (G)
 - b) Pyrimidine bases (1 ring) – cytosine (C) and thymine (T) [RNA T replaced by uracil (U)]
 - c) Complimentary base pairs linked by weak H bonds A=T (double bond) G≡C (triple bond) A=U (double bond).
- 3) A phosphate group (PO_4^{2-})

Structure of DNA (Watson and Crick 1953)

- Phosphate sugar backbone on outside of double helix
- Complimentary bases are oriented towards central axis
- Each strand is a compliment of the other e.g. if the sequence on one strand is TATTCCGA the sequence on the other strand is ATAAGGCT.
- DNA also has a secondary structure of bends and twists.

DNA replication – when a cell divides each daughter cell is given a replica of the DNA

- Beginning at a particular point (the origin) the double strand is separated like a zipper or a tuning fork using a special enzyme. Each strand serves as a template.
- A *DNA polymerase* enzyme binds to each strand and moves from base to base generating a complimentary strand.

- Each double stranded DNA molecule that is generated has one strand from the parent molecule and one newly synthesized strand.

RNA (three types – messenger mRNA, ribosomal rRNA, and transfer tRNA) structure is similar to DNA but it is single stranded and contains U rather than T

Gene Expression - Transcription of DNA – transcribe – DNA and RNA speak the same language

- DNA separates and the code on one section of the DNA (the gene) is used as a template to form a complimentary single strand of RNA
- Action of *RNA polymerase* – similar to DNA replication but entire molecule is not reproduced.
- DNA molecule closes back up behind the point where transcription occurs.
- When *RNA polymerase* reaches the end of the gene being transcribed, the RNA molecule breaks away.

Translation – change from the DNA language to the language of proteins

- Messenger RNA now contains the coded information for the sequence of amino acids for a protein.
- mRNA migrates to the ribosome, which contains rRNA and protein
- mRNA positions itself on the ribosome (the work bench) and tRNA shuttles amino acids to the ribosome where they are matched to the transcribed message on mRNA.
- The ribosome moves along mRNA molecule until the entire protein molecule is synthesized.

Regulation – cell doesn't waste energy producing proteins it doesn't need

- Constitutive genes – expressed all the time
- Induced genes – expressed in response to a trigger – e.g. the presence of a particular substrate will trigger the cell to produce enzymes to degrade that substrate.

Microbial Genetics Continued

Genetic exchange never obligatory in prokaryotes - most bacteria in a colony are genetically identical.

Mutation - some of the daughter cells may differ slightly in genetic makeup.

- Low probability of mutation (can accelerate with exposure to mutagens, UV)
- High growth rate
- Natural selection

Example - *E. Coli* probability of mutation $\sim (10)^{-7}$ per cell division however $2(10)^{10}$ are produced daily in the human colon. Result is 2,000 mutants per day - if specific mutation makes an organism more fit it will out-compete its neighbors.

Genetic Exchange

- Transformation: DNA from lysed cells are taken up and incorporated into living cells DNA
- Transduction: genetic exchange mediated by viruses.
 - Some viruses (e.g. HIV) combine their DNA (or DNA copies of their RNA) with the host DNA during a dormancy period. A stimulus triggers the virus to “take over” the host’s reproductive machinery and the host starts packaging up viral DNA. Some of the host’s DNA can be taken up along with the virus DNA.
- Conjugation : transfer of a plasmid from a donor to a recipient.

Microbial Interactions

Microorganisms rarely exist as pure cultures, however, pure culture models have been used extensively to learn about microbial populations by microbiologists. We’ll attempt to scratch the surface regarding microbial interactions.

Definitions:

- Positive interaction: increases growth rate
- Negative interaction: decreases growth rate

Interactions within a single population

- Cooperation: cycling leaking metabolites, increasing concentration of extracellular enzymes, shielding from toxins.
- Genetic exchange: requires minimum density
- Competition for substrates
- Formation of toxic metabolites

Neutralism: Populations have different metabolic requirements or are spatially separated.

Positive Interactions between populations

- Commensalism: one population benefits one is unaffected
 - Obligate anaerobes benefit if aerobes deplete O₂ supply
 - Growth factors leaked from one cell benefit another
 - Dissolution of insoluble compounds by pH decrease
 - Product of metabolism (H₂S, CH₄) is substrate for another group.
- Synergism (protocooperation): both populations benefit, not obligate.
 - Cross feeding: two groups can degrade a compound neither has the enzymes to degrade alone.
 - Supplying growth factors for each other, nutrient cycling, detoxification.
- Mutualism: both organisms benefit, obligate. Four types:
 - Extracellular facultative: can separate the organisms. Example: Lichens: algae or cyanobacteria + fungus.
 - Extracellular obligate: example ruminant and anaerobe.
 - Intracellular: one organism lives within the cell of another, both benefit. Example paramecium and chlorella.
 - Organelle. Chloroplasts of green plants, mitochondria of eukaryotes.

Negative interactions between populations

- Competition: for carbon, oxygen, nutrient, etc.
 - Competitive exclusion principle: two populations can not occupy the same niche because the population with the highest growth rate will win the competition.
 - Two niches can be spatially separated.
 - You can have population shifts depending on the conditions.
- Ammensalism: one population produces a compound that inhibits another : antibiotics, oxygen, alcohols, acids
- Parasitism: parasite derives nutritional benefit from host. Long period of contact.
 - Viruses and microorganisms
 - Microorganisms in plants and animals
- Predation: more rapid than parasitism, predator is usually bigger than prey.
 - Predator may benefit the prey by keeping the population from exhausting the resources, feeding them, removing weak members from the population.

Stoichiometry and Bacterial Energetics (see Chapter 2 R&M)

Mass balance approach - for a given quantity of waste, determine the amount of nutrients, electron acceptor, etc. that must be supplied. Determine the amount of products and cell biomass that will be formed.

Generalized reaction:

Carbon source + energy source + electron acceptor + nutrients → microbial cells + end products

McCarty (1975) technique - write a quantitative expression for the stoichiometric equation no matter what the carbon, energy, or electron acceptor is (limited to chemotrophic metabolism)

When microorganisms use an electron donor for growth, a portion of the electron donor is used for cell synthesis and a portion is used for energy production.

Rules:

1. The reactions consist of three components:

R_c - reaction to form cell material

R_d - reaction of the electron donor

R_a - reaction of the electron acceptor

2. All reactions are written on an electron equivalent basis (1 electron transferred)

3. All reactions are written as reductions

4. The total reaction is:

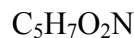
$$R = -R_d + f_e R_a + f_s R_c$$

f_e = fraction of electron donor used for energy

f_s = fraction of electron donor used for synthesis

$$f_e + f_s = 1 \quad (2.36)$$

Empirical formula for cell material - based on elemental analysis, however highly variable, depends on physiological state



Accounting for P: P (in grams) = grams of N required for cells/5

Chemical reaction for electron donor (see Tables 2.2 and 2.3):

Known inorganic compound (NH_4^+ , Fe^{2+}) - write half reactions

Known organic compound (methanol, benzoate) - write half reactions

Wastewater - Use average value - $C_{10}H_{19}O_3N$

Determine from elemental analysis $C_nH_aO_bN_c$

Determine from determine from COD, N, carbohydrate, protein, lipid content (see original papers for details).

Chemical reaction for electron acceptor (see Table 2.4) O_2 , NO_3^- , SO_4^{2-} , Fe(III), CO_2

Chemical reaction for cell synthesis (see Table 2.4) - Depends on N source - NH_4^+ , NO_3^- , NO_2^- , N_2 (less synthesis with assimilatory N reduction or N_2 fixation since these are expensive processes in terms of energetics)

Determination of f_s :

From cell yields (Y) under experimental conditions:

$$f_s = 1.42Y_n = Y_n \frac{gVSS}{gCOD} \times \frac{8gCOD}{eq} \times \frac{eq}{5.65gVSS}$$

where Y_n is the net cell yield (g cells produced/gram COD consumed) $Y_n = \frac{dX/dt}{-dS/dt}$.

From energetic relationships - the greater the energy from a substrate the greater yield.

$$\Delta G_r = \Delta G_r^o + RT \ln \left[\frac{[C]^c [D]^d}{[A]^a [B]^b} \right] \cong \Delta G_a^o - \Delta G_d^o$$

Pyruvate is used as a common intermediate for cell synthesis. First we compute the free energy change required to convert the carbon source to pyruvate (ΔG_p):

$$\Delta G_p = 35.09 - \Delta G_c^o \quad (2.45)$$

for heterotrophic bacteria the carbon source is the electron donor (see table 2.3 for ΔG_c^o) for autotrophic bacteria, $\Delta G_p = 113.8 \text{ kJ/e}^- \text{ eq}$.

Next, pyruvate is converted to cell carbon. This value will vary depending what the nitrogen source is. If ammonium is used as an N source, $\Delta G_{pc} = 18.8 \text{ kJ/e}^- \text{ eq}$, for nitrate, $\Delta G_{pc} = 13.5 \text{ kJ/e}^- \text{ eq}$.

A certain amount of energy is lost to heat. The efficiency is given as ϵ . The energy required for synthesis (ΔG_s) is therefore:

$$\Delta G_s = \frac{\Delta G_p}{\epsilon^n} + \frac{\Delta G_{pc}}{\epsilon} \quad (2.46)$$

the value of n (+1 or -1) accounts for the fact that for some substrates energy is obtained when they are converted to pyruvate (if ΔG_p is positive n = +1).

A value A is defined representing the amount of electron donor that must be oxidized to produce one equivalent of cells:

$$\frac{f_e}{f_s} = A = - \frac{\frac{\Delta G_p}{\epsilon^n} + \frac{\Delta G_{pc}}{\epsilon}}{\epsilon \Delta G_r} = \frac{\Delta G_s}{\epsilon \Delta G_r} \quad (\text{note sign error in text}) \quad (2.48)$$

The maximum value of f_s, f_s^o , is computed from A by: $f_s^o = \frac{1}{1+A}$ (2.49)

Note that f_s^o does not consider the energy required for maintenance.

Microbial Growth

- ⇒ Growth: an increase in the number of cells in a population.
- ⇒ Cell growth: binary fission. Growth of an individual cell continues until the cell divides into two new cells.
- ⇒ Population growth. Add cells to a nutrient medium and measure cell numbers (direct counts, optical density, VSS, viable counts, etc) as a function of time. See figure of typical growth curve.
- ⇒ I Lag phase. At first it appears as though no growth is occurring. What's happening?
- Manufacture of required enzymes or other cell constituents
 - Enzyme induction: metabolic control operates in such a way that some enzymes aren't produced unless they're needed.
 - Induced enzymes: requires the presence of an inducer to be produced. Negative control: a compound (repressor) binds to the DNA at an operator and prevents transcription of that section of the genetic code that codes for that enzyme. If the substrate is present, it binds with the repressor and allows transcription to occur.
 - Constitutive enzymes: key enzymes required under many different conditions.
 - Growth may be occurring but is insignificant.
 - Long variable lag times may indicate that genetic mutations or exchange may be required before growth can occur.
- ⇒ II Exponential Growth. Log growth phase
- Each cell divides to form two new cells, which in turn divide to form two new cells, etc. Doubling time can vary from 20 min. to several days.
 - Balanced growth. Increased in biomass is accompanied by a proportional change in all properties of the culture (RNA, DNA, protein, etc.)
 - Mathematical expression for exponential growth:
$$\frac{dX}{dt} = \mu X$$
$$\text{at } t = 0 \text{ } X = X_o$$
$$X = X_o e^{\mu t}$$
 - Generation time (g):

$$\frac{X}{X_0} = 2$$

$$\ln(2) = \mu g$$

⇒ III Unbalanced growth phase – population starts to feel stress. Cell size may be decreasing, cellular components may be synthesized at unequal rates:

- Decrease in storage polymers
- Increase in lipids (cell membrane) due to smaller cells.

⇒ IV Stationary phase – cells switch to starvation survival mode.

- Use up stored polymers
- Form endospores.
- Normal place for organisms in the environment.
- Growth rate = death rate

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

$$\mu X = bX$$

$$\frac{dX}{dt} = 0$$

⇒ V Death phase or endogenous phase.

- Depletion of nutrients
- Increase in toxins

$$\frac{dX}{dt} = -bX$$

Effects of various conditions on growth

⇒ Effect of substrate concentration

- Monod Experiment: perform a series of growth experiments at varying substrate concentrations, S, determine μ based on exponential growth phase of each experiment. Plot μ vs. S. Empirical equation (Monod equation):

$$\mu = \frac{\hat{\mu}S}{K_s + S}$$

- has been observed for N, P, Fe, carbon sources, in pure and mixed cultures

⇒ Cell growth and substrate utilization

$$r_g = \frac{dX}{dt} = \mu X = \frac{\hat{\mu}XS}{K_s + S} = -Yr_{ut} = -Y \frac{dS}{dt}$$

- Y = yield coefficient is dependent on:
 - How much energy is available from e^- donor and acceptor
 - Degree of polymerization of substrate, presence of growth factors, vitamins.
 - Metabolic pathway
 - Physical parameters of system: mixing, surfaces.
 - Nitrogen source.
- Expression for rate of substrate utilization

$$r_{ut} = \frac{dS}{dt} = -\frac{\hat{\mu}XS}{Y(K_s + S)} = -\frac{\hat{q}XS}{K_s + S}$$

where $\hat{q} = \hat{\mu} / Y$

⇒ Effect of endogenous metabolism

$$r_d = -bX$$

$$\text{net growth rate: } r_{net} = \frac{\hat{\mu}XS}{K_s + S} - bX = -Yr_{ut} - bX$$

⇒ Simplifications of Monod expressions

- Low substrate concentration $S \ll K_s$

$$\frac{dS}{dt} = -k_1 S \quad \text{where } k_1 = \frac{qX}{K_s}$$

- High substrate concentration $S \gg K_s$

$$\frac{dS}{dt} = -k_o \quad \text{where } k_o = qX$$

⇒ Multiple limiting nutrients

$$\text{Interactive model: } \mu = \hat{\mu} \left(\frac{S_1}{K_{S1} + S_1} \right) \left(\frac{S_2}{K_{S2} + S_2} \right)$$

$$\text{Non-interactive model: } \mu = \min \left[\left(\frac{\hat{\mu}_1 S_1}{K_{S1} + S_1} \right), \left(\frac{\hat{\mu}_2 S_2}{K_{S2} + S_2} \right) \right]$$

⇒ Effect of pH

- Most bacteria grow best at pH 6-8
- Some are particularly pH sensitive (e.g. nitrifiers): $\mu_A = \hat{\mu} [1 + 10^{(6.5 - pH)}]^{-1}$
- Some are acidophiles

⇒ Inhibitory substrates

- For some compounds, the growth rate decreases at high S

$$\text{Andrew's equation: } \mu = \frac{\hat{\mu} S}{K_s + S + S^2 / K_I} \text{ where } K_I \text{ is the inhibition coefficient}$$

⇒ Other inhibitors (e.g. heavy metals, other organics)

⇒ Effect of temperature

- Arrhenius type equation does not predict a decrease in activity at high temperature

$$k_1 = k_2 \theta^{(T_1 - T_2)}$$

⇒ Thresholds: not enough substrate present to induce enzymes or support growth.

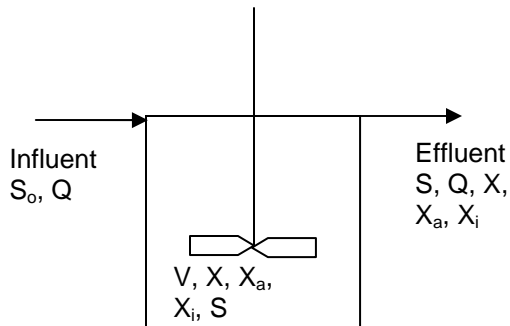
⇒ Diauxy; the compound that gives the higher growth rate is used first. May also be due to physical/ chemical properties (e.g. solubility)

⇒ Enhancement of degradation: second compound may increase the population density.

⇒ Comatabolism: primary substrate induces necessary enzymes for fortuitous degradation of a cometabolite.

Continuous growth of microbial cultures

- ⇒ Chemostat: reactor used for continuous growth of microbial cultures. Engineers would call it a CFSTR (continuous flow stirred tank reactor).
- ⇒ Mass balance on active biomass, X_a :



Rate of accumulation = mass flow rate in – mass flow rate out ± net generation

$$\frac{d(X_a V)}{dt} = QX_o - QX_a + Vr_{net}$$

$$r_{net} = \mu X_a - bX_a = \frac{\hat{\mu} X_a S}{K_s + S} - bX_a$$

- ⇒ Assumptions: Monod kinetics describe degradation

- Soluble substrate
- Single limiting substrate
- Constant Q
- Completely mixed system

$$0 = -\frac{QX_a}{V} + \frac{\hat{\mu} X_a S}{K_s + S} - bX_a$$

$$\frac{1}{\theta} = \frac{Q}{V} = \frac{\hat{\mu} S}{K_s + S} - b = r_{net} / X_a$$

- ⇒ Mass balance on substrate, S.

$$\frac{d(SV)}{dt} = QS_o - QS - Vr_s$$

$$r_s = \frac{\mu}{Y} X_a = \frac{\hat{\mu} X_a S}{Y(K_s + S)}$$

$$\frac{Q(S_o - S)}{V} = \frac{S_o - S}{\theta} = \frac{\hat{\mu} X_a S}{Y(K_s + S)} = r_s$$

⇒ Active biomass density in reactor: depends on S_o , Y and residence time.

$$X_a = \frac{Y(S_o - S)}{1 + \theta b}$$

⇒ Outlet substrate concentration controlled by θ and kinetics, not a function of S_o .

$$S = \frac{K_s(1 + \theta b)}{\theta(\hat{\mu} - b) - 1}$$

⇒ Observed Yield: ratio of biomass density observed to substrate used:

$$Y_{obs} = \frac{X_a}{(S_o - S)} = \frac{Y}{1 + \theta b}$$

⇒ Minimum outlet concentration: take the limit as $\theta \rightarrow \infty$

$$S_{min} = \frac{K_s b}{\hat{\mu} - b}$$

⇒ Washout point: residence time so low that the cells washout before there is time for any reaction to occur:

$$\theta_{min} = \frac{K_s + S_o}{S_o(\hat{\mu} - b) - K_s b}$$

CFSTR with Biomass Recycle: Activated Sludge

⇒ Parts of the A-S process:

- Aeration Basin: completely mixed aerobic reactor with diffused or mechanical aeration. MLSS – wastewater mixed with active biomass.
- Clarifier: quiescent basin. Cells are separated by sedimentation.
 - Removes MLSS to meet SS requirements.
 - Concentrates solids for return to bioreactor.
- Solids recycle: return activated sludge (RAS). A portion of the cells are returned to the aeration basin.
 - Selective pressure for growth of cells with good settling characteristics.
 - Maintain high X in MLSS.
- Solids wasting: waste activated sludge (WAS). A portion of the solids are wated each day, either from the recycle flow or from the aeration basin.

⇒ Assumptions for modeling:

- Aeration basin is a CFSTR
- $X_0 = 0$
- Biodegradation occurs only in aeration basin
- Monod kinetics: single limiting soluble substrate.

⇒ Key concept: SRT > HRT.

- Long SRT – low effluent substrate concentration
- Low HRT – small reactor volume, high throughput, system economy.

⇒ Mass balance on microbial biomass results in:

$$\frac{r_{net}}{X_a} = \frac{Q_w X_w}{V X_a} = \frac{1}{\theta_c} = \frac{\hat{\mu} S}{K_s + S} - b$$

- Inverse sludge age is equal to net specific growth rate.

$$S = \frac{K_s(1 + \theta_c b)}{\theta_c(\hat{\mu} - b) - 1}$$

- $S = f$ (kinetic parameters and SRT)
- Same as for CFSTR except replace HRT with SRT

⇒ Mass balance on substrate yields:

$$\frac{S_o - S}{\theta} = \frac{\hat{\mu} X_a S}{Y(K_s + S)} = r_{ut}$$

$$X_a = \left(\frac{\theta_c}{\theta} \right) \frac{Y(S_o - S)}{1 + b\theta_c}$$

$$X_v = \frac{\theta_c}{\theta} \left[X_i^o + \frac{1 + (1 - f_d)b\theta_c}{1 + b\theta_c} Y(S_o - S) \right]$$

- Substrate utilization rate defined the same as for CFSTR
- Biomass density depends on both SRT and HRT.
- Text discusses effect of biomass debris on reactor. Debris adds to total MLSS in aeration basin. As we increase the SRT we increase the debris concentration in the bioreactor. One reason we can't infinitely recycle.

⇒ Observed Yield:

$$Y_{obs} = \frac{\text{biomass wasting rate}}{\text{mass of substrate removed from reactor}} = \frac{Y}{1 + \theta_c b} \text{ based on active fraction only}$$

⇒ Sludge production:

$$r_w = Q_w X_w = \frac{X_v V}{\theta_c}$$

⇒ Rate of O₂ utilization (does not account for mass transfer):

- when S is expressed as UBOD (or COD) and r_w expressed as g VSS/day:

$$R_o = Q(S_o - S) - 1.42r_w$$

- recycle ratio $\alpha = \frac{Q_r}{Q} \approx \frac{X}{X_w - X}$

- F/M ratio: $F/M = \frac{\text{mass loading of COD}}{\text{total VSS in bioreactor}} = \frac{QS_o}{VX_v} = \frac{S_o}{\theta X_v}$

- Sludge volume index: $SVI = \frac{\text{mL of solids after 1/2 hour of settling}}{\text{total solids in grams}}$

Activated Sludge Variations

⇒ Conventional Plug-flow with recycle

- Influent and RAS are added at one end and mixed liquor exits at the other end:

$$\frac{1}{\theta_c} = \frac{\hat{\mu}(S_o - S)}{(S_o - S) + (1 + R)K_s \ln(S_i / S)}$$
$$S_i = \frac{S_o + RS}{1 + R}$$
$$R = \frac{Q_r}{Q}$$

- More efficient than a CFSTR: higher influent concentrations leads to higher reaction rates
- True plug-flow usually not achieved
- Doesn't handle shock loads as well as CFSTR
- θ_c 5-15 days; θ 4-8 hours
- BOD loading 20-40 lbs. BOD₅/1000 ft³-day
- MLSS 1500-3000 mg/L

⇒ Modifications of PFR

- Tapered Aeration – provide more aerators near the inlet
- Step-feed aeration – even out organic loading by introducing influent at several locations
 - Reduced bioreactor requirements
 - More complex operation
 - Reduced nitrification
- Extended aeration: θ_c 2-30 days, θ 18-36 hours, MLSS 3,000-6,000 mg/L, low F/M
 - Organisms in endogenous phase
 - High O₂ requirements, bulking problems common
 - Low solids production, good nitrification
 - Typically used in small communities, package plants in camps, rest areas, resorts, etc.
- Modified Aeration (high rate)
 - Opposite of extended aeration short θ_c 0.2-0.5 days, short θ 1.5-3 hours
 - Used as rough pre-treatment (e.g. industrial pre-treatment programs) or first stage of a two stage system (e.g. A-S followed by TF).
- Contact stabilization
 - Two compartments: one to treat wastewater, one to stabilize solids

$$\theta_c = \frac{X_c V_c + X_s V_s}{X_w Q_w} \text{ since } X_s \text{ is so high total } V \text{ is lower for same } \theta_c$$

- Takes advantage of the sorption of non-soluble organics onto sludge
- Organisms from the sedimentation basin enter reaeration tank at high solids concentration (4,000-9,000 mg/L)
- Colloidal material adsorbed to sell surfaces and metabolized
- High endogenous decay in reaeration tank, then hungry organisms contact wastewater in aeration basin
- Can reduce aeration requirements by up to 50%
- Good modification of existing facilities.

⇒ High Purity O₂

- Use pure O₂ instead of air for oxygenation of wastewater
- Greater driving force for mass transfer (C*_{air} = 8-9 mg/L, C*_{O₂} = 30-40 mg/L)
- Covered reactors
- Gases collected from headspace and used to aerate the next reactor.
- Good for high O₂ demand wastewaters, upgrades of existing facilities.

⇒ Oxidation Ditch

- oval channel, mechanical aerators, good mixing, good nitrification/denitrification
- long θ_c 10-30 days, long θ 8-36 hours

⇒ Sequencing Batch Reactors

- Wastewater treated in batch process
- Fill: ~ 1 hr., react ~ 4 hour, settle ~ 1-2 hour, draw ~ ½ hour, idle ~ variable.
- Single tanks: small communities, industrial treatment processes, soil-slurry systems
- Multiple tanks: continuous process with a lot of flexibility
- Aerobic/anoxic operation
- Research/ teaching

⇒ Aerated lagoons: CFSTR w/out recycle

- Large earthen basins with mechanical aerators on pontoons.
- Common for industrial pretreatment, small communities, upgrades to oxidation ponds.
- Low cost, technically simple, large land requirements, no solids recycle, algae

⇒ Membrane bioreactor process - membrane separation system replaces clarifier.

- Two types - series or submerged
- Very high loading rates, MLSS (up to 20 g/L) and SRT (5-20 d)
- No clarifier - smaller, 100% solids removal, process efficiency independent of clarifier.
- Very high quality effluent (no solids, low BOD, nutrient removal configs possible).

Bulking in Activated Sludge Processes

- ⇒ Growth of filamentous organisms needs to be balanced with non-filamentous growth.
 - Enough filaments to hold the floc together
 - Too much interferes with settling, foaming problems occur
 - SVI 150 mL/g – good balance
- ⇒ Why filaments form:
 - Low concentrations of substrates: C, N, P, DO, trace elements
 - High surface/volume ratio organisms dominate under low substrate conditions
 - Inputs of reduced sulfur compounds may favor the growth of filaments
 - Low K_s organisms: high substrate affinity. These organisms usually not competitive under high substrate conditions:
- ⇒ Strategies for control of filamentous organisms
 - Plug flow reactor: conventional A-S. Organisms go through an area of the reactor with high substrate concentration. Natural selection for organisms with high growth rate under high substrate conditions.
 - Add chlorine or H_2O_2 to return WAS. Low K_s organisms more susceptible to low concentration disinfectants. Emergencies only.
 - Remove TRS compounds from influent using stripping or oxidants.
 - Addition of nutrients (N,P), trace elements, growth factors. May be needed with industrial wastes.
 - Improved aeration: maintain $\sim 2\text{mg/L}$ in aeration basin.
 - Improved reactor hydraulics: get rid of zones where you have long residence times.
 - Use of a selector:
 - Short residence time reactor (10-30 min) with high F/M and sufficient aeration.
 - Metabolic selector: some floc forming organisms are facultative – can grow under anoxic or anaerobic conditions – while most filamentous organisms are not.
 - Zone with NO_3^- as e^- acceptor.
 - Anaerobic zone

SRT as a master variable

- Effects soluble substrate removal
 - Effects particulate organic substrate removal
 - Effects bioflocculation
 - Effects nitrification
 - Effects sludge production
 - Effects O₂ requirements
- ⇒ Selection of operating SRT depends on objectives
- Must be above minimum SRT
 - $[\theta_{cmin}]_{lim} = \frac{1}{\hat{\mu} - b} \approx \frac{1}{\hat{\mu}}$ below $[\theta_{cmin}]_{lim}$ washout occurs
 - Depends on treatment objectives
 - Soluble organic substrate removal (high $\hat{\mu} \Rightarrow$ low θ_C)
 - Particulate organic substrate removal (moderate $\hat{\mu} \Rightarrow$ moderate θ_C)
 - Nitrification (low $\hat{\mu} \Rightarrow$ high θ_C)
 - Must be greater than minimum SRT for process stability (SF > 1.5)
 - Must allow for bioflocculation
 - Must meet O₂ and sludge production objectives

Selection of MLSS concentration

⇒ Total mass of MLSS not concentration controls performance. Selection of bioreactor volume determines concentration.

⇒ Once SRT is selected, biomass total mass is fixed:

$$X_B V = \frac{Q \theta_C Y (S_o - S)}{1 + b \theta_C}$$

⇒ MLSS 2,000-5,000 mg/L. Minimum MLSS concentration needed for flocculation.

⇒ Large MLSS concentration \Rightarrow small bioreactor volume \Rightarrow large clarifier needed

⇒ Oxygen transfer and mixing: $V_L < V < V_U$

- V_L bioreactor volume below which, the power required for O₂ transfer will result in floc shear
- V_U bioreactor volume above which, the power required for mixing is greater than the power requirements for O₂ transfer.

Gravity Separation of Biological Slurries

⇒ Critical link between the treatment process and effluent discharge.

⇒ If clarifier is not properly designed – high solids in effluent

⇒ Two functions of clarifier:

- Separation of solids
- Thickening sludge

⇒ Typical design values:

- Overflow rates (O/F = Q_o/A) 12-40 m/d for conventional A-S
- Solids flux ($G_T = (Q+Q_r)X/A$) 70-140 kg/(m²-d) average flow and < 220 kg/(m²-d) peak.
- Weir loadings - keep small particles from being swept over weirs (for circular clarifiers $WL = Q_o/\Sigma\pi d_w$) 100-150 m²/d at average flow and < 375 m²/d at peak.

⇒ Experiment: take a graduated cylinder and put in some MLSS at an initial solids concentration X_o and watch the contents. After some time different zones develop:

- Type I settling: discrete settling - follows Stoke's law.
- Type II settling: flocculent settling – particles coalesce and increase in mass and settle at a faster rate.
- Type III settling: hindered or zone settling – inter particle forces hinder settling, particles remain in a fixed position relative to each other.
- Type IV settling: compression – structure is formed and liquid moves up through the particle interstices.

⇒ Two areas must be looked at for design: area required for clarification, and area required for thickening. Calculate both and then use larger for design. Depth of the clarifier usually 12-20 ft.

⇒ Area required for clarification:

$$v_x \geq \frac{Q - Q_w}{A_{CL}} \quad (1)$$

- Settling velocity of solids at concentration X_o must be greater than the overflow rate or you'll wash the cells over the weirs.

⇒ Area required for thickening:

Flux across a boundary = solids flux due to gravity settling + solids flux due to underflow

$$G_T = X_i v_i + X_i \left(\frac{Q_u}{A} \right) \quad (2,3)$$

$$G_T = G_s + G_U$$

- How to determine G_s ? Plot interface height vs time for various values of X_o : $G_s = X_i v_i$

- Plot G_s vs X : at very low concentrations, velocity is independent of concentration. At very high concentrations, settling velocity approaches zero. A maximum flux is observed at an intermediate concentration.
- Plot solids flux vs X due to underflow: $G_u = X_i (Q_u/A)$. Note this requires that you pick a value for A , if this value is too low, you start the procedure over. R&M gives a simple method that does not require iterations.
- Plot total solids flux vs X and determine limiting solids flux:
 - At some point in the clarifier $X = X_c$ (the critical solids concentration)
 - At that point, the solids mass can't get through any faster. Therefore the limiting flux must be greater than the applied flux or you'll overflow your system:

$$G_c \geq \frac{(Q + Q_r)X}{A} \quad (4)$$

- Simple graphical approach:

Plot H vs. t for each X

Determine v_x for each X

Plot G_s vs X

Determine initial design X_w for your A-S system

Draw a line from X_w to the vertical axis tangent to the solids flux curve.

The line intercepts the vertical axis at G_c

Use Equation 4 to determine A