Physical Treatment

1. Flow Measurement

- Not a unit operation
- Important to decide (1) chemical additives (2) air volume (3) recycle rates, etc.
- Measurement : open channel venturi meters mechanisms

2. Screening

- First unit operation (mostly!!!)
- Removal of large subjects (sticks, rags, boards, etc.) in WW
- Uniform size particles for further treatment : screenings $\rightarrow$ Landfill, anaerobic treatment
- Primary purpose : To protect pumps & other mechanical equipments
  To prevent cloggings of valves
- Manual/Mechanical cleaning : Headloss
  Clean system (0.1 m), less than 0.3m preferable

(1) Bar Racks

- Removal of coarse subjects
- Usually, 1 cm apart
- Headless calculation

$$h_L = \frac{1}{0.7}\left(\frac{V^2 - v^2}{2g}\right)$$

h_L : headloss [m]
0.7 : an empirical discharge coefficient to account for turbulence
V : velocity of flow through the openings of the bar rack [m/s]
v : approach velocity in upstream channel [m/s]
g : acceleration due to gravity [m/s^2]

(2) Screens

- Removal of fine subjects (Sometimes, used for effluent from secondary treatment processes)
- Disk/Drum type

- Headloss calculation

$$h_L = \frac{1}{C\,(2g)} \left(\frac{Q}{A}\right)^2$$

h_L : head loss [m]

C : coefficient of discharge for the screen (≅ 0.60)

g : acceleration due to gravity [m/s^2]

Q : discharge through screen [m^3/s]

A : effective open area of submerged screen [m^2]

3. Comminuting

- Size reduction of screenings (≤ 8mm in dia.)
  1. Communitor type : rotaing teeth
  2. Barminutor : vertical bar rack + cutting head traveling up & down the rack
- High maintenance item in WWTP
- Small plant: Bypass through
- Large plant: several comminutors in parallel

4. Grit Removal (Chamber)

- Grit?: (in)organic solids & metal fragments (ex. Pebbles, sand, egg shells, glass, bone chips, seeds, coffee & tea grounds, etc.)
- Accelerated wear on pumps & sludge handling devices
- Deposits in pipes & clarifiers
- Mostly refractory → occupy sludge digester volume
- Basics: providing an enlarged channel area where reduced flow velocities allow grit to settle out
- Hydraulically: designed to remove particles (0.2 mm dia. & specific gravity of 2.65) by type 1 settling
- Horizontal velocity: steady flow at 0.3 m/s
- Design trend: aerated grit chamber
  - → for lighter organics in suspension
  - → should be designed based on peak flow
  - → 15-20 min. aeration

5. Sedimentation (Settling)

- A unit operation designed to **concentrate and remove suspended (organic) solids** from the ww
- Device: Clarifier
- Important step as a primary treatment operation
- Similar to the operation of clarifying water for portable supplies
- Should be designed not to produce any gas
- 4 types of sedimentation : possible to have all 4 occuring simultaneously

(1) Discrete particle sedimentation (Type 1)

- sedimentation of particles in a suspension of low solids concentration
- particles settle as individual entities & no significant interaction with neighboring particles
- Ex: sedimentation of grits and sand particles from wastewater

(2) Flocculant Sedimentation (Type 2)

- particles that coalescing (or flocculating) during the sedimentation operation
- particles $\rightarrow$ mass increase $\rightarrow$ settle at a faster rate
- used to remove chemical flocs

(3) Hindered (or Zone) Sedimentation (Type 3)

- interparticle forces are sufficient to hinder the settling of neighboring particles
- particles tend to remain in fixed positions with respect to each other $\rightarrow$ the mass of particles settles as a unit

(4) Compression Sedimentation (Type 4)

- by the weight of the particles
- usually occurs in the lower layers of a deep settling facilities (ex. sludge thickening facilities)

- Think about sludge settling in a cylinder!!!

- When you design a settling facility, you should be able to calculate (1) settling velocity, (2) % removal, (3) area (vol.) required → **Overflow (hydraulic loading) rate**

6. Flotation

- Separating solid or liquid particles (ex. grease) from a liquid phase
- To remove very small or light particles that settles slowly (may be advantageous over settling operation)
- Operation: Introducing fine gas (usually air) bubbles into the liquid phase → bubbles + particles → Buoyant force → Removed by a skimming operation
- For a better efficiency → use of chemicals (i.e., aluminum, activated silica etc.)

(1) Dissolved Air Flotation (DAF)

- Air is dissolved in the ww under a high pressure → release of the pressure (to the atmospheric level)
- small systems : pressurizing the entire flow (to 275-350 *kPa*)
- larger units: recycling a portion of the effluent → pressurizing (& semisaturated with air) → mixed with the (unpressurized) main stream → the air finally comes out of solution

(2) Air Flotation

- Direct injection of the air into the ww stream
- Aeration alone for a prolonged period may be required

(3) Vacuum Flotation

- ww + air (saturation) → vacuum application → dissolved air to come out of solution as fine bubbles

(4) Chemical Additives

- Addition of chemicals (i.e., aluminum, ferric salts, activated silica,organic polymers etc) to aid the flotation process
- Creating a surface or a structure for absorbing (entrapping) air bubbles

7. Filtration

- Passing ww through a stationary bed of granular medium that retains solids in the ww
- → physical straining of particles too large to pass between filter grains
- Transport mechanisms (i.e., flocs in ww to filter media): settling, inertial impaction, diffusion of colloids, Brownian movement (random, thermal motion of solute due to continuous bombardment by the solvent molecules) etc.
- Usually a polishing step to remove small flocs or precipitant particles
- **Downflow**, upflow, biflow, pressure, and vacuum filtration
- Cleaning: Mostly backwash
- Changing: by headloss calculation (see handout)
- Filter media: silica, garnet sands, anthracite coal, etc.

(1) Slow Sand Filter

- First filter to be used for water purification
- Use of fine sand with an effective size of about 0.2 mm
- Large space requirement and capital intensive
- Ineffective for highly turbid ww: rapid surface plugging → frequent cleaning

(2) Rapid Sand Filter

- To alleviate difficulties of the slow sand filter
- Sizes range : 0.35 to 1.0 mm or larger, with effective sizes of 0.45-0.55 mm

(3) Dual Media Filters

- Mostly, anthracite coal + silica sand
- Removal of large particles and flocs by anthracite layer & most of the smaller material by sand layer
- Advantage: effective use of pore space → in hope of better performance
- Disadvantage : Any sudden increase in hydraulic loading removes particles from the anthracite layer (held rather loosely in the anthracite layer) → transports them to the surface of the sand layer → rapid binding at this level → decrease in

efficiency

(4) Mixed Media Filters

- Ideal type (in case of downflow) : medium sized granules graded evenly from large at the top to small at the bottom → by using three or more types of media with carefully selected size and density
- Mixed (or dual) media filters → direct filtration of water of low turbidity without settling operations.

Chemical Treatment (Disinfection)

- Operations aimed at killing pathogens (not to destroy all living organisms (i.e., sterilization))
- Must be toxic to pathogens at concentrations well below the toxic thresholds to humans
- U.S.: chlorination, Europe: ozonation

1. Chlorination

- Types of chlorine applied to water:

Cl_2 (gas) + H_2O → H^+ + HOCl

$Ca(OCl)_2$ (solid) → Ca^{2+} + $2OCl^-$

NaOCl (solid) → Na^+ + OCl^-

(HOCl: hypochlorous acid, $Ca(OCl)_2$: calcium hypochlorite, NaOCl: sodium hypochlorite, OCl: hypochlorite ion)

- Cost: $Ca(OCl)_2$ and NaOCl > liquefied Cl_2
- However!!!, (1) Cl_2 gas is a very strong oxidant that is toxic to humans
  (2) Since it is heavier than air, it spreads slowly at ground level
  → **extreme** care must be provided in its manufacture, shipping, and use

- Disinfection mechanisms
  (1) Low concentrations: by penetrating the cell and reacting with the enzymes
  (2) Higher concentrations: oxidation of the cell wall
- Factors affecting the process;
  (1) Forms of chlorine
  (2) pH
  (3) Temperature
  (4) Concentration
  (5) Contact time
  (6) Types of organism

2. Ozonation

- Ozone can be produced in a high strength electrical field from pure oxygen or from the ionization of clean and dry air.

$$O_2 \xrightarrow{\text{high voltage}} O + O$$
$$O + O_2 \leftrightarrow O_3$$

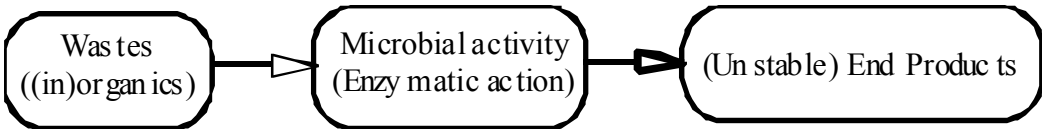
- A powerful oxidant which reacts vigorously with bacteria and viruses $\rightarrow$ more effective than chlorination
- Must be produced on-site and used immediatelybecause it is chemically unstable $\rightarrow$ 2-3 times expensive than the cost of chlorination (but dechlorination is not required)

3. Ultravilet Irradiation

- Most effective band: 2000 to 3000 $\mathring{A}$ for both bacteria & viruses
- Promising method but unproven (in U.S.)
- Glass surface must be constantly cleared

Biological Treatment of Wastewater

Simple(?) Mechanism



Some Important Terms in Biological WW Treatment

Metabolisms

The sum total of all the chemical and physical changes that occur in a living system.

1. Anabolism: synthetic rxn in a living system
2. Catabolism: degradative rxn with the generation of ATP in a living system
3. Amphibolic: Both ATP & biosynthetic building blocks formed

In Microbial Degradation

1. Transformation: anabolic or catabolic change in a compound of interest
2. Mineralization: the ultimate recycling of an organic molecule to its mineral constituents (always includes CO_2 & sometimes CH_4)
3. Refractory: hard to be biodegradable
4. Recalcitrant: not transformed or mineralized under any conditions tried to date
5. Xenobiotic: "Foreign to biology". Not synthesized by biological processes.
6. Cometabolism: metabolism by a MO of a compound that the MO is unable to use as a **sole** source of energy or an **essential** nutrient
7. BTEX: Benzene, Toluene, Ethylbenzene, Xylene

Enzymes

- Biological catalysts made of **protein** (Coenzyme: nonprotein substances, or groups associated with enzyme which are necessary for enzymatic activity)
- Generally, enzymes shows catalytic specificity
- Enzymatic reactions have **pH & Temperature optimum**

Habitat & Niche

1. Habitat: Physical location where an organism is found (microhabitat for MO)
2. Niche: (A broader concept than habitat) The physical habitat + **the functional role** + interactions of the MO within that space

Mutation

- Any change in the sequence in the genome
- Natural rate of mutation ≈ 10^{-8} → 10^{-3} by strong mutagens

HRT: Hydraulic Retention Time

SRT: Solid Retention Time

Engineer's Point of View for MO in WW Treatment

→ The **"We don't care what it does as long as it gets done"** approach

Classification of Microorganisms by Structure & Metabolic Types

I. Procaryotic and Eucaryotic MO

A. Procaryote: simple internal cellular architecture; no membrane bound nucleus
   1. Eubacteria
      a. Includes most known bacteria
      b. Includes cyanobacteria ("blue-green algae")
      c. Cell wall characteristics divide group into Gram positive (G^+) & negative (G^-) types
      d. Cell chemistry is similar to eucaryotes
   2. Archaebacteria
      a. Includes methanogens, halophiles, thermoacidophiles
      b. Distinctive cell chemistry including membrane lipids with branched hydrocarbon chains joined to the polar region by ether bonds

B. Eucaryote: complex internal cellular architecture; membrane bound nucleus
   1. Unicellular, coenocytic[1] or mycelial[2] ; little or no cell or tissue differentiation

Protists: algae, fungi, protozoa

2. Multicelluar; extensive differentiation of cells and tissues

   Plants & animals

1: coenocyte: a multinucleate organism that lacks cell walls

2: mycelium(-ia): the vegetative structure of a fungus that consists of a multinucleate mass of cytoplasm, enclosed within a branched network of filamentous tubes known as hyphae)

II. Viruses: Intracellular parasites of both eucaryotic and procaryotic organisms

A. Generally smaller (much smaller) than bacteria

B. Commonly have a protein coat (capsid) surrounding nucleic acid (Deoxyribonucleic acid (DNA) or Ribonucleic acid (RNA)) core

C. May have membrane surrounding capsid

D. Virus may reproduce and cause host cell to lyse or DNA (from virus itself or produced from virus RNA) may become incorporation into host cell DNA

III. Classification of MO by Metabolic Types

A. Classification by **energy source**:

1. Light energy:

   Phototrophs → all photosynthetic organisms including bacteria (ex. cyanobacteria) and algae

2. Chemical energy:

   a. **Chemoorganotrophs (Heterotrophs)**: Energy from preformed organic matter → many of bacteria, fungi, protozoa

   b. **Chemolithotrophs**: Energy from oxidation of mineral compounds → only some bacterial species

B. Classification by **carbon source**

1. **Heterotrophs**: C from organic matter → bacteria, fungi, protozoa

2. **Autotrophs**: C from CO_2 → some bacteria, algae

3. **Photoorganotrophs**: C from both CO_2 and simple organic compounds → some bacteria, algae

IV. Classification of MO by Structure

A. Bacteria

a. Rods: bacilli

b. Spheres: cocci

c. Spirals: sprilli

d. Kidney, comma shaped

e. Pleomorphic: change shape depending on environmental conditions or growth stage

B. Fungi

Filaments, single cells, colonies

V. Classification of MO by Oxygen Utilization or Tolerance

A. Aerobic

Oxygen tolerated and used as an electron acceptor (O_2 reduced to H_2O) at the end of e^- transport chain in metabolism

B. Microaerophilic

Sub-atmospheric concentrations of O_2 tolerated and use as an e^- acceptor.

C. Aerotolerant

O_2 - not toxic but not used in metabolism.

D. Facultative

Capable of using either aerobic or fermentative metabolism depending on the availability of O_2 → Many bacteria & some fungi (ex. brewery yeast)

F. Anaerobic

1. Anaerobic respiration: essentially same biochemistry as aerobic metabolism but an alternative e^- acceptor (e.g., NO_3^-, SO_4^{2-}, etc.) is used.

2. Fermentation: No e^- transport chain involved in metabolism. Organic matter undergoes a balanced series of oxidation and reduction reactions. Organic acids and alcohols are major products.

Catabolism of Organic Materials

A. Carbohydrates degradation

· Glycolysis

Polymers ⟶ Monomers (mostly glucose) ⟶ Pyruvate ⟶ Lactate

⟶ Ethanol

⟶ Acetyl-CoA

B. Fat degradation

· β-oxidation

Fat molecules ⟶ Long chain fatty acids ⟶ Acetyl-CoA (C_2)

+

Propionyl-CoA (C_3)

C. Protein degradation

· Hydrolysis of peptide bonds by (i) proteinases and (ii) peptidases

Higher structure ⟶ Amino acids ⟶ Deamination

(tertiary/quaternary) (primary)

MOs only utilize soluble forms of (in)organics inside the cell!!!

A. BIOLOGICAL PROCESSES

(1) Aerobic Process

The goals of the aerobic biological process is oxidation of organic material and ammonium (NH_4^+) or ammonia (NH_3). These reactions are brought about with oxygen as the final electron acceptor. Organic material is mineralized to H_2O, CO_2, NH_4^+ or NH_3, and other constituents, and the NH_4^+ or NH_3 are oxidized to nitrate (NO_3^-). Each reaction also results in the synthesis of new cell mass.

(I) Biochemical Reactions

These are the approximated reactions.

(i) Oxidation & Synthesis

$$CHONS + O_2 + \text{nutrient} \rightarrow CO_2 + NH_3 + C_5H_7NO_2 + H_2O + \text{other end product}$$

(ii) Nitrification

$$NH_3 \xrightarrow{\textit{Nitrosomonas}} NO_2^- \xrightarrow{\textit{Nitrobacter}} NO_3^-$$

(iii) Denitrification

$$NO_3^- \longrightarrow NO_2^- \longrightarrow NO \longrightarrow N_2O \longrightarrow N_2$$

(iv) Endogenous respiration

$$C_5H_7NO_2 + 5O_2 \rightleftarrows 5CO_2 + 2H_2O + NH_3 + \text{energy}$$

(113) (160)

1 : 1.42

(II) Microbiology

Achromobacter, Beggiatoa, Flavobacterium, Geotirichum, Nitrobacter, Nitrosomonas, Pseudomonas, Sphaerotilus are the common microorganisms found in aerobic systems.

Protozoa such as *Vorticella, Opercularia, Epistylis* and rotifers do not stabilize waste, but they consume dispersed bacteria and small biological floc particles that have not settled (i.e. protozoa and rotifers act as effluent polishers)

(i) Suspended growth system

Bdellovibrio, Lecicothrix, Mycobacterium, Nocardia, Thiothrix, Zoogloea are bacteria that are

mostly found in suspended growth systems.

(ii) Attached growth system

Alcaligenes, Chlorella, Fusazium, Mucor, Penicillium, Phormicium, Sphaerotilus natans, Sporatichum, Ulothrix, Yeasts are mostly found in attached growth systems. Algae such as *Chlorella, Phormicium, Ulothrix* are not directly involved in waste stabilization. They add oxygen to the system but they can cause clogging of the system, which produces odors.

(2) Anaerobic Process

The anaerobic process mineralize organic and inorganic materials in the absence of molecular oxygen. Because oxygen is toxic to most anaerobic microorganisms, molecules other than oxygen such as sulfur or carbon dioxide are used as the final electron acceptors. Due to the extreme difficulty of isolation of anaerobes and the complexity of the bioconversion processes, much still remains unsolved about anaerobic processes compared to the aerobic process. The overall anaerobic conversion of biodegradable organic materials to the final end products, CH_4 and CO_2, is believed to proceed in three stages which occur simultaneously.

(I) Biochemical Reactions

(i) Hydrolysis phase

Large insoluble molecules such as carbohydrate, protein and fat are solubilized into their monomer. This process is mediated by extracelluar enzymes produced by various microorganisms called hydrolyzing bacteria. This step is often called the liquefaction phase.

(ii) Acidification phase

A group of microorganisms called acidogens, which produce mainly acids, convert the soluble products of the hydrolysis phase to short chain organic acids, CO_2 and hydrogen. These acids normally have chain lengths from C_1 to C_4. This step is often called the acidogenic phase or acidogenesis.

(iii) End products formation

Finally, methane producing bacteria, called methanogens, convert these short chain organic acids to final products which are mainly CH_4 and CO_2. This step is called the methanogenic phase or methanogenesis.

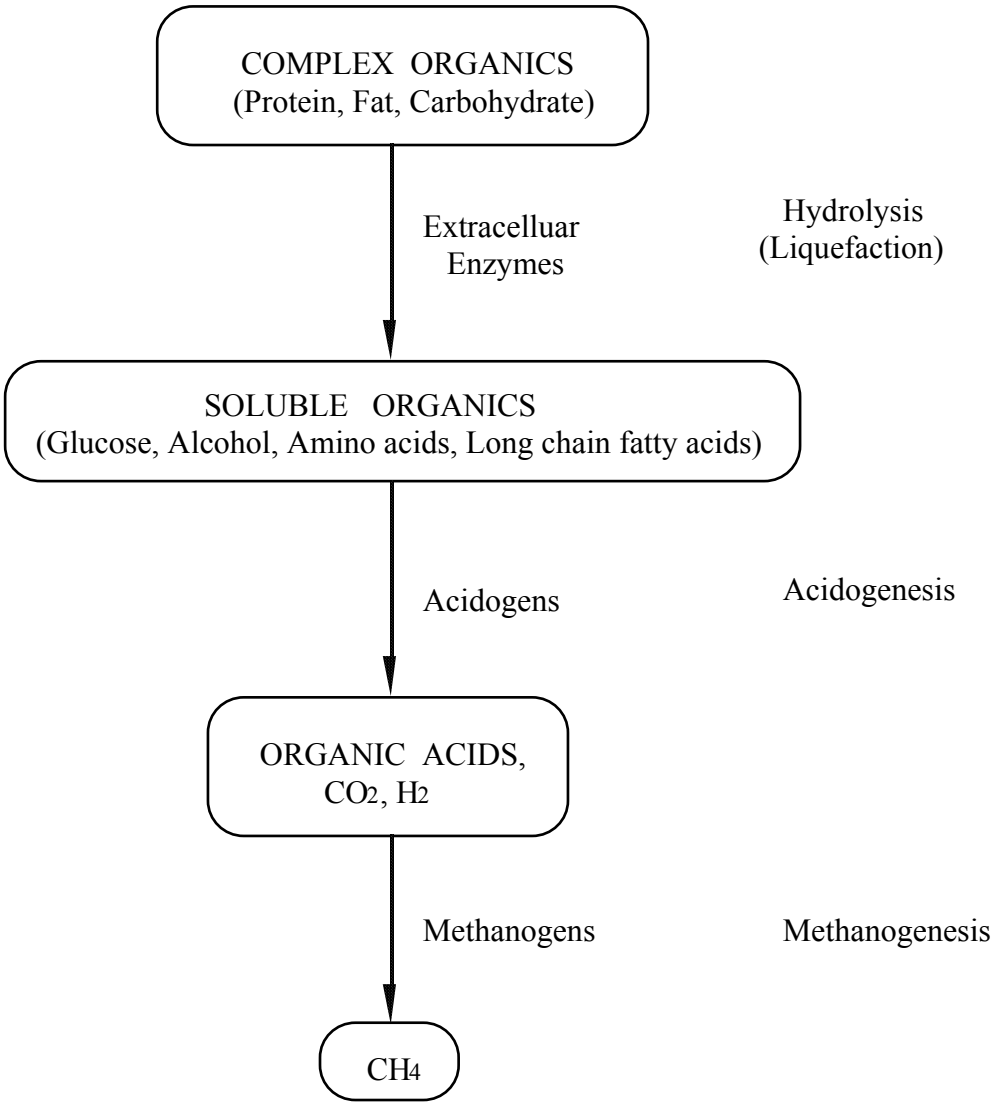
(II) Microbiology

(i) Hydrolyzing bacteria and acidogens

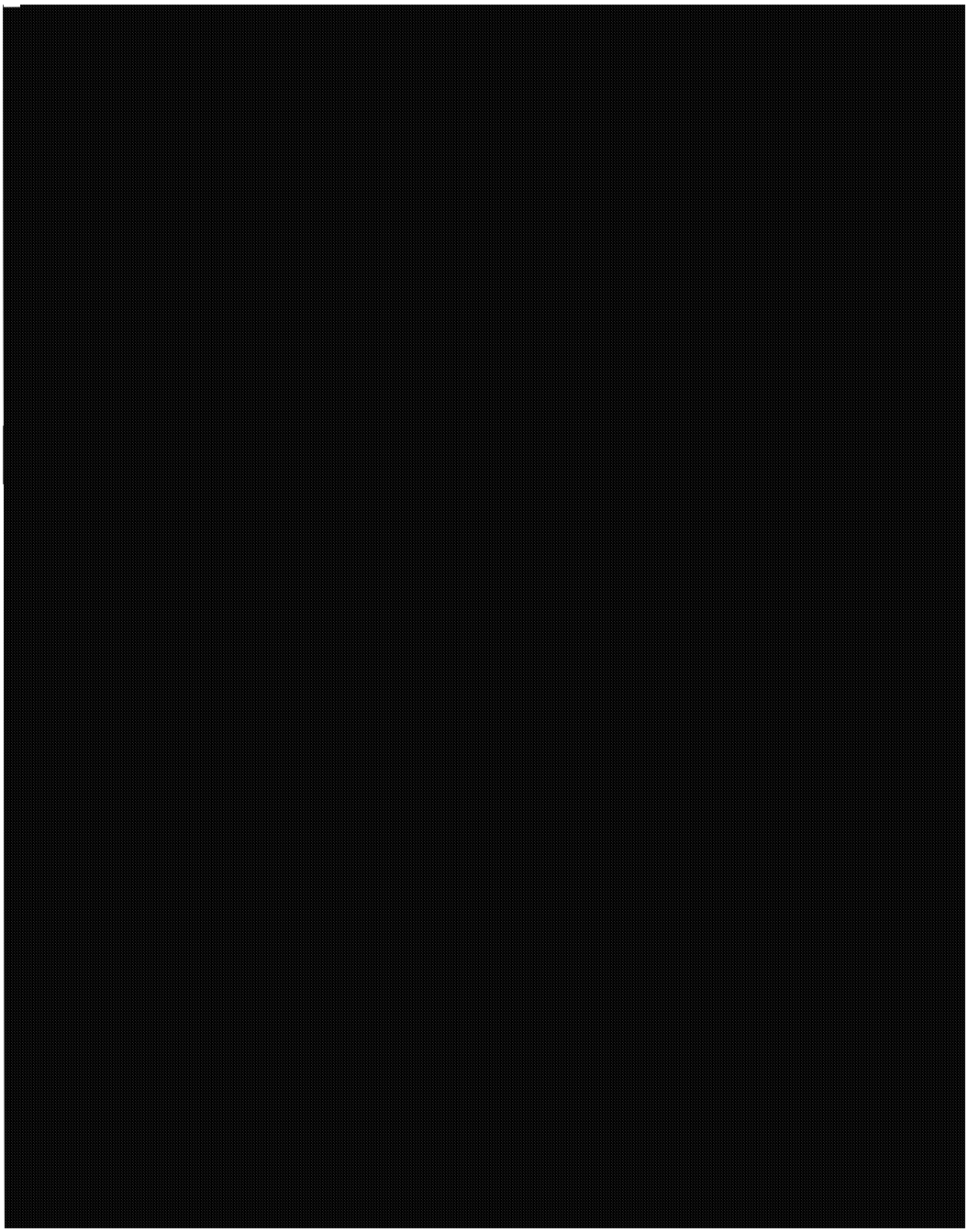
Actinomyces, Bifidobacterium spp., *Clostridium* spp., *Corynebacterium* spp., *Desulphovibrio* spp., *E. coli, Lactobacillus, Peptococcus anaerobus, Staphylococcus*

(ii) Methanogens

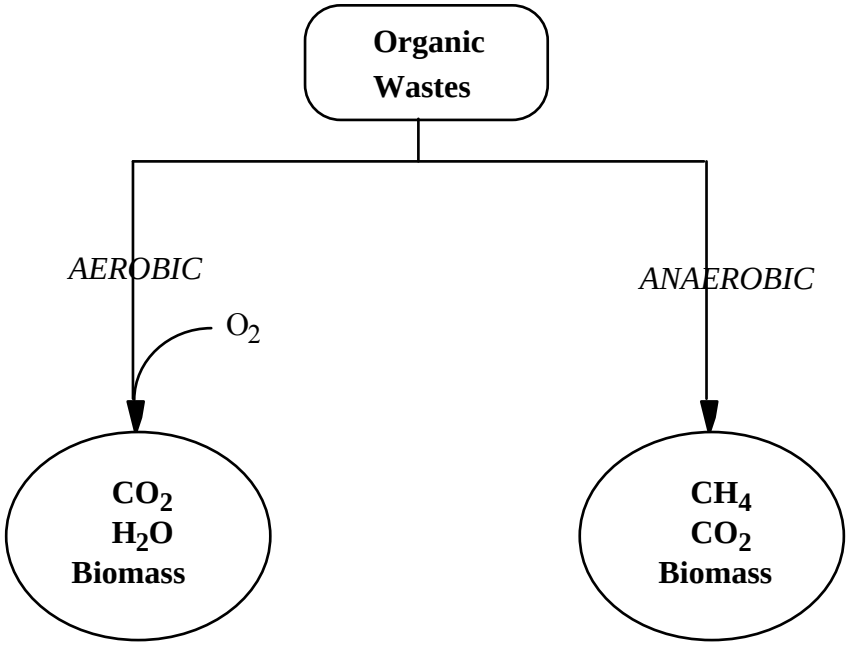
Methanobacillus, Methanobacterium, Methanococcus, Methanosarcina



Schematic diagram of overall pathway of anaerobic metabolism



The pathway of anaerobic degradation of lactose



General differences in the pathway and the end products between aerobic and anaerobic processes.

B. BASIC CONCEPTS OF BIOKINETICS

Many kinetic models for biological wastewater treatment have been developed during the last 40 years. They are very useful for the understanding of the treatment process and for the comprehension of applicabilities and limitations. In many cases, development of mathematical models for the process is of great importance to evaluate further developments and operational conditions in the right way. The design of full-scale treatment facilities today should be based on kinetic models derived from experimental and operational data.

(1) Microbial Growth

The change of biomass in a microbial culture undergoing a balanced growth generally follows the first order model. The rate of growth at any time is proportional to the number or mass of microorganisms present in the system at the time as described by the following equation:

$$r_g = \left(\frac{dX}{dt}\right)_g = \mu X \qquad (1)$$

Several models have been developed that indirectly establish a value of μ. The most widely accepted of these is the Monod equation. This equation assumes that the rate of biomass production is limited

by the rate of enzyme reactions involving utilization of the substrate compound that is in shortest supply relative to its need. Eq. (2) shows this relationship.

$$\mu = \frac{\mu_m S}{K_s + S} \tag{2}$$

Eq. (2) shows that specific growth rate is a hyperbolic function of the substrate concentration. The Monod equation also indicates that μ can have any value between zero and μ_m, provided that the substrate concentration can be held constant at a given value of μ.. Any system designed for the continuous cultivation of microorganisms meets this condition.

(2) Substrate Utilization

For some increment of time, the change in the substrate concentration is proportional to the concentration of biomass present as described by the following equation:

$$r_{su} = \left(\frac{dS}{dt}\right)_{su} = qX \tag{3}$$

Lawrence and McCarty presented an equation (4) that related the rate of substrate utilization to both the concentration of substrate and of microorganisms, and is as follows:

$$r_{su} = \frac{kXS}{K_s + S} \tag{4}$$

When applying Eqs. (2) and (4) to any microbial ecosystem, substrate concentration surrounding microorganisms is an important consideration for evaluating kinetic parameters. The carbon and energy source, as measured by ultimate biochemical oxygen demand (BOD_u), chemical oxygen demand (COD), or total organic carbon (TOC), is usually considered to be the growth-limiting substrate in biological wastewater treatment processes.

(3) Microbial Yield

If all the substrate were converted to biomass, then the rate of biomass production would equal the rate of substrate utilization. However, the rate of biomass production is less than the rate of substrate utilization since catabolism converts some part of substrate into nongrowth factor. Thus:

$$r_g = Yr_{su} \tag{5}$$

The factor Y varies depending on the metabolic pathway used in the conversion process. Aerobic

processes are more efficient than anaerobic processes with respect to biomass production and have a larger value for Y. The substrate concentration usually decreases as microorganisms grow. Therefore, the following equation can be developed.

$$r_{su} = -\frac{1}{Y}\frac{\mu_m SX}{K_s + S} \qquad (6)$$

(4) Microbial Decay

Under certain conditions such as cell lysis, presence of predators, and endogenous metabolism, microorganisms lose the ability to grow or to subdivide. Such morbid microorganisms die, resulting in a decrease in biomass population. To account for this phenomenon, it is assumed that the rate of biomass decrease is proportional to the concentration of biomass in the system.

$$r_d = \left(\frac{dX}{dt}\right)_d = k_d X \qquad (7)$$

(5) Kinetic Model Development

Kinetic models are usually developed by writing material balances describing the mass rate of change in substrate and in biomass. Mass balance around the system will be:

Biomass (X): Net change = Input - Output + Growth - Decay

Substrate (S): Net change = Input - Output - Utilization

C. BASIC TYPES OF REACTORS AND BIOKINETICS

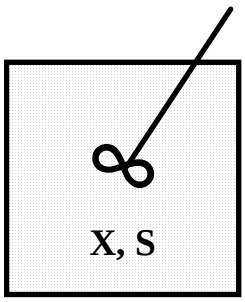
(1) Batch Reactor

Batch reactors are widely used in many industries, especially in handling relatively small volume productions of very expensive materials such as enzymes, medicines and etc., because the same batch reactor can be used to produce a variety of products. Sequencing batch reactors (SBR) have also been proposed for wastewater treatment. This batch or sequencing batch process is still used in some very small installations, although it is rare.

In batch reactor experiments, the reactor is filled with the reactant(s) and brought to the desired reaction conditions. Then, during the reaction, samples are analyzed and the concentration is recorded against time. When more than one reactant is involved, it is necessary to repeat the experiment with different initial compositions. In a batch reactor, there is no flow, instead a batch of material is placed into a vessel, inoculated, and microbial growth and substrate utilization occur. As growth proceeds, reaction conditions change and consequently so does the growth environment. The

microorganisms present at different times will be in different physiological conditions and no steady state is possible, which makes modeling the batch system much more complicated.

Either the differential or the integral method must be used with data obtained from a batch reactor because the data are in the form of concentration versus time and do not provide a direct measure of the reaction rate as a function of concentration.



Biomass (X): Net change = Input - Output + Growth - Decay

$$\left(\frac{dX}{dt}\right)_{net} V = 0 - 0 + (r_g - r_d)V$$

$$\left(\frac{dX}{dt}\right)_{net} = (\mu - k_d)X$$

$$\frac{X_{t+1} - X_t}{\Delta t} = \left(\frac{\mu_m S_t}{K_s + S_t} - k_d\right)X_t$$

$$X_{t+1} = \left\{1 + \left(\frac{\mu_m S_t}{K_s + S_t} - k_d\right)\Delta t\right\} X_t \quad \rightarrow \text{ numerical analysis !!!}$$

Substrate (S): Net change = Input - Output + Utilization

$$\left(\frac{dS}{dt}\right)_{net} V = 0 - 0 + r_{su} V$$

$$\left(\frac{dS}{dt}\right)_{net} = r_{su}$$

$$= - \frac{\mu_m}{Y} \frac{SX}{K_s + S}$$

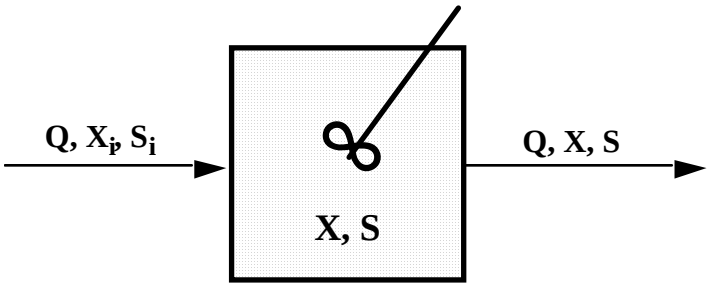
$$\frac{S_{t+1} - S_t}{\Delta t} = - \frac{\mu_m}{Y} \frac{S_t X_t}{K_s + S_t}$$

$$S_{t+1} = \left(1 - \frac{\mu_m}{Y} \frac{X_t}{K_s + S_t} \Delta t\right) S_t \quad \rightarrow \text{ numerical analysis !!!}$$

(2) Continuous Stirred Tank Reactor (CSTR)

A CSTR, also known as continuous flow stirred tank reactor (CFSTR) or completely mixed reactor, is used very frequently in many industrial fields. It is usually equipped with baffles and a mixer which is operated at a sufficiently high speed so that the mixing is assumed to be perfect. It is assumed to be homogeneous and instantaneous so that any reactant carried into the reactor by the feed is dispersed evenly throughout the reactor without any time delay. In addition, the reaction is assumed to take place only in the reactor so that the effluent composition is the same as the reactor composition.

The most often used method to get mathematical expression of biokinetics is steady state operation, where feed is supplied continuously until a steady state is achieved. Then, effluent concentration is recorded, and another steady state run should begin by changing the feed concentration and/or the feeding rate. Thus a number of steady state runs are required to obtain data relating reaction rate to concentration whereas a single unsteady-state run may be used to gain the same information from a batch reactor. By varying independent variables such as flow rate and/or influent substrate concentration, it is possible to solve mathematical expressions experimentally.



Biomass (X): Net change = Input - Output + Growth - Decay

$$\left(\frac{dX}{dt}\right)_{net}V = 0 - QX + (\mu - k_d)XV$$

at steady state, $\left(\frac{dX}{dt}\right)_{net} = 0$ & divided by X,V

$$\mu - k_d = \frac{QX}{VX} = \frac{Q}{V} = \frac{1}{\tau} \qquad \rightarrow \ (\tau\text{: hydraulic retention time (HRT)})$$

$$\mu = \frac{1}{\tau} + k_d = \frac{1 + k_d \tau}{t} = \frac{\mu_m S}{K_s + S}$$

$$S = \frac{K_s(1 + k_d \tau)}{(\mu_m - k_d)\tau - 1}$$

Substrate (S): Net change = Input - Output + Utilization

$$\left(\frac{dS}{dt}\right)_{net} V = QS_i - QS + r_{su} V$$

$$\left(\frac{dS}{dt}\right)_{net} = \frac{Q}{V}(S_i - S) + r_{su}$$

at steady state $\left(\frac{dS}{dt}\right)_{net} = 0$

$$-r_{su} = \frac{Q}{V}(S_i - S) = -\frac{\mu X}{Y}$$

$$\frac{Q}{V} = \frac{1}{\tau} \text{ and } \mu = \frac{1}{\tau} + k_d$$

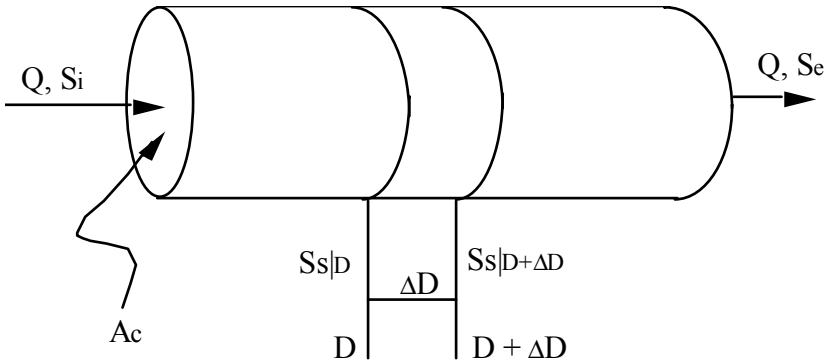
$$X = \frac{Q}{V}\frac{Y(S_i - S)}{\mu}$$

$$= \frac{Y(S_i - S)}{1 + k_d \tau}$$

(3) Plug Flow Reactor (PFR)

Fluid containing the substrate continuously passes through the reactor and effluent is discharged in the same sequence in which it enters. This type of flow is similar to that in long tubes or tanks with a high length to width ratio. For an ideal PFR, the flow pattern inside has uniform velocity and concentration in the radial direction at any point along the length of the reactor while longitudinal (axial) dispersion is minimal or absent.

In the ideal PFR, concentration of substrate and biomass continuously varies with time and the distance. In other words, it is not only necessary to know how the concentration varies with time but also how it varies along the length of the reactor.



Substrate (S): Net change = Input - Output + Utilization

$$\frac{\partial S}{\partial D} A_c \Delta D = Q(Ss|D) - Q(Ss|_{D+\Delta D}) - r_{su} A_c \Delta D$$

$$\frac{\partial S}{\partial D} A_c = \frac{Q(Ss \mid_D - Ss \mid_{D+\Delta D})}{\Delta D} - r_{su} A_c$$

if $\Delta D \rightarrow 0$

$$\frac{\partial S}{\partial D} A_c = Q \frac{\partial Ss}{\partial D} - r_{su} A_c$$

at steady state, $\frac{\partial S}{\partial D} = 0$

$$\frac{dS}{dD} = \frac{A_c}{Q} r_{su}$$

if it follows first order kinetics $r_{su} = kS$

$$\int_{Si}^{S} \frac{dS}{S} = k \frac{A_c}{Q} \int_o^D dD$$

$$\ln \frac{S_i}{S} = k \frac{A_c D}{Q} = k \frac{V}{Q} = k \tau$$

$$S = S_i e^{-kt}$$

The material balance for biomass is similar.

(4) Suspended Growth Systems

These are the systems in which microorganisms responsible for the waste stabilization are

maintained in suspension within the liquid.

(5) Attached Growth Systems

These are the systems in which microorganisms responsible for the waste stabilization are attached to various inert media such as rocks, sands or artificial materials. These systems are also called fixed film processes.

D. VARIOUS BIOLOGICAL TREATMENT SYSTEMS

(1) Aerobic Suspended Growth Systems

(I) Aerobic Activated-Sludge Process

This process usually employs a CSTR followed by a settling device where the cells are separated from the treated stream and was so named because it involved the production of an activated microorganisms capable of stabilizing wastes aerobically. Various versions of this process have been proposed but they are fundamentally similar.

The aerobic condition is maintained by the injection of air or pure oxygen or mechanical aeration, which also maintains the reactor content in a completely mixed regime. A portion of separated cells in the settling device are recycled to the reactor and a portion is wasted to maintain proper biomass concentration. The level of biomass concentration in the reactor depends on the treatment efficiency and microbial growth. The biomass concentration in the effluent could be high if organisms such as *Thiothrix, Actinomycetes, Beggiatoa, Sphaerotilus, E. coli*, and fungi are present in high concentration inside the reactor because these filamentous microorganisms don't settle well. This is known as "Bulking".

(II) Plug Flow with Recycle

This is a PFR equipped with a recycle system to maintain proper biomass concentration. The particles retain their identity and remain in the tank for a time equal to the theoretical detention time. The true plug-flow-recycle system is more efficient in stabilization of most soluble wastes than the CSTR system. In practice, a true plug flow regime is difficult to obtain because of longitudinal dispersion.

(III) Aerated Lagoons (Ponds)

This process is basically the same as the activated sludge process, except that an earthen basin is used for the reactor, and the oxygen required by the process is supplied by surface or diffused aerators. The large surface area of an aerated lagoon can cause more significant temperature effects than are normally encountered in the conventional activated sludge process.

(IV) Sequencing Batch Reactor (SBR)

A SBR is a fill-and-draw activated sludge system. The unit processes of SBR and conventional activated sludge systems are identical except that in SBR operation the processes are carried out sequentially in the same tank, whereas the conventional activated sludge process is carried out continuously. A wastewater treated by conventional activated sludge process can be treated with SBRs.

The SBR systems have five steps: (1) fill → (2) react (aeration) → (3) settle (sedimentation/clarification) → (4) draw (decant) → (5) idle

(V) Aerobic Digestion

Aerobic digestion is an alternative method of treating the organic sludges produced from various treatment operations. As the supply of available substrate is depleted, the microorganisms begin to consume their own cell materials (protoplasm) to obtain energy for cell maintenance reactions. Cell tissue is aerobically oxidized to CO_2, water, and ammonia. Actually, about 75-80% of the cell tissue can be oxidized: the remainder is composed of inert components and nonbiodegradable (recalcitrant) materials. The ammonia from this oxidation is subsequently oxidized to nitrate as digestion proceeds.

Biochemical reaction:

$$C_5H_7NO_2 + 5O_2 \rightarrow 5CO_2 + 2H_2O + NH_3 + \text{energy}$$
$$(113) \quad (160)$$
$$1 \quad : \quad 1.42$$

(2) Aerobic Attached Growth Systems

(I) Trickling Filter

The trickling filter consists of a bed of a highly permeable medium to which microorganisms are attached and through which wastewater is trickled. The filter media usually consist of either small rocks or various plastic packing materials. Filters are constructed with an underdrain system for collecting the treated wastewater and any biological solids detached from the media. The collected liquid is passed through a settling device where the biomass is separated from the treated wastewater.

Microorganisms attached to the filter media utilize the organic material present in the wastewater. As the microorganisms grow, the thickness of the slime layer increases, and the diffused oxygen is consumed before it can penetrate the full length of the slime layer. Thus, an anaerobic environment is established near the surface of the media. As the slime layer increases in thickness, the absorbed organics are metabolized before it can reach the microorganisms near the media surface resulting in no available organic substrate. Microorganisms near this area lose their activities and lose the ability to cling to the surface. The liquid flow then washes the slime off the media and new

microorganisms start to grow on that surface. This loss of the slime layer is called "sloughing" and is dependent on the organic and hydraulic loading on the filter.

In operation, the bed is filled with wastewater and the wastewater is allowed to contact the media for a short time. The bed is then drained and allowed to rest before the cycle is repeated. A typical cycle is 12 hours of which 6 hours is for operation and 6 hours is for resting. A portion of the liquid collected in the underdrain system (or settled effluent) is recycled, usually to dilute the strength of the incoming wastewater and to maintain the biological slime layer in a moist condition.

The first trickling filter was placed in operation in England in 1893.

Limitations:

(1) high incidence of clogging

(2) long rest period is required

(3) low loading rate.

(II) Rotating Biological Contactor (RBC)

A RBC consists of a series of closely spaced circular disks, which are submerged in wastewater and rotated through it. Microorganisms are attached to the surfaces of the disks and form a slime layer over the surface area of the disks. The rotation of the disks alternately contacts the biomass with the organics in the wastewater and then with the atmosphere for adsorption of oxygen. The rotation provides for oxygen transfer to the biomass and removal of excess biomass from the disks by shearing forces.

(III) Packed Bed Reactors

A packed bed reactor consists of a container (reactor) that is packed with a medium to which the microorganisms can become attached. Wastewater is normally introduced from the bottom of the container through an appropriate underdrain system or inlet chamber. The flow patterns, however, can be upflow or downflow.

(3) Anaerobic Suspended Growth Systems

(I) Anaerobic Contact Process

The fundamentals of this process are the same as for the activated sludge process except it is operated under anaerobic condition. The wastes are digested in a CSTR with recycling unit, which is sealed off from the entry of air. After digestion, the mixture is separated in a clarifier or settling facility, and the supernatant is discharged as effluent. Settled anaerobic biomass is then recycled to the reactor to maintain proper microbial population. Because of the low growth rate of anaerobic microorganisms, the excess sludge that must be disposed of is minimal. This process has been used successfully for the treatment of high strength organic wastes including food and agricultural

wastewater.

(II) Upflow Anaerobic Sludge Blanket (UASB) Process

The UASB process is a recently developed anaerobic process which is based on the slow upward movement of waste through dense bed and blanket zones of biologically active sludge. Basically, the reactor consists of three distinct zones: the sludge bed, sludge blanket, and settling/biomass separation zones.

The sludge bed zone is responsible for 80 to 90 % of the waste stabilization occurring in the reactor while occupying roughly 30 % of the reactor volume. This main waste stabilization is due to high biomass concentration in the sludge bed. Under favorable conditions for sludge granulation, anaerobic granules with high microbial activities and excellent settling characteristics, up to 3-4 mm in diameter, are formed in the reactor. The next zone encountered by the waste stream is the sludge blanket zone, which occupies about 50 % of the total reactor volume and contains less sludge concentration than the sludge bed zone. The sludge in the blanket zone has almost uniform particle size and originates from the bed where it is whirled up by rising gas bubbles. A third area is a zone in the settler where the sludge concentration decreases to a minimum. The fluid flow in the settling zone is laminar, which might be described as a plug-flow region.

The main function of the separator at the top of the reactor is to drive the rising gas and biomass particles in toward the gas collector, where a swirling action occurs, and the biomass settles back down into the reactor, thereby preventing most of the biomass rising with gas bubbles from leaving the reactor.

(III) Anaerobic Sludge Digestion

This is one of the oldest methods of decomposing concentrated sludges, normally produced from aerobic processes. This can be processed in any type of airtight reactor but a CSTR is widely used. Sludge is introduced continuously or intermittently into the reactor and is retained for a time to achieve the necessary level of treatment. Organic and pathogen contents are reduced during operation and the treated sludge is withdrawn continuously or intermittently.

The two types of commonly used reactors are standard and high rate. In the standard rate process, the contents inside the reactor are not heated and mixed. In high rate process, the contents are heated and mixed completely. The retention time of the standard rate process is about 30 - 60 days while the retention time for the high rate process is typically 15 days or less.

(4) Anaerobic Attached Growth Systems

(I) Anaerobic Filter Process

The process uses a column filled with various solid media providing binding sites for anaerobic microorganisms. The wastewater normally flows upward through the column, contacting the media

on which anaerobic bacteria grow and are retained. Because the microorganisms on the media are resistant to washing off, a long biological retention time can be achieved with short HRT, so the anaerobic filter can be used for the treatment of low strength wastes at ambient temperature.

(II) Anaerobic Expanded Bed Process

In the expanded bed process, which sometimes called a fluidized bed reactor, the wastewater is passed upward through a bed of appropriate media such as sands and coals on which microorganisms can attach and grow. Effluent may be recycled to dilute the incoming waste and to provide an adequate flow to maintain the bed in an expanded condition. Because a large biomass can be maintained, the expanded bed process can be used to stabilize various wastes at very short HRT.

Factors Effecting Microbial Activity

Microorganisms, particularly bacteria, are able to perform the recycling of organic and inorganic matter in nature because they exist in a wide variety of species with different metabolic requirements and capabilities. Most microbiologists believe that all naturally occurring materials and all but a very few synthetic materials are subject to microbial attack.

The activities of microorganisms, from the human viewpoint, are either useful or harmful. Indeed, the same activity may be useful or harmful depending on the environment in which it happens. The unexpected growth of lactic acid bacteria such as *Streptococcus lactis* in milk, for example, irritates those who drink milk, but the growth of this same bacteria in milk under controlled conditions is the basis of manufacturing processes in the dairy industry.

Many environments are capable of supporting the growth of many different microbial species, and it is within such environments that the balance of species may shift frequently and rapidly due to relatively small changes in nutrient supply or physical conditions. While it would be ideal to study microorganisms under natural conditions, there will be little information about their characteristics and activities without subjecting them to different experimental conditions. Due to their small size, direct observation is not possible; nor is it possible by observing the entire population to determine which of the species comprising a mixed microbial population is responsible for any single activity. Therefore, it is desirable to study mixed populations of microorganisms under controlled conditions. This allows researchers to study their interactions or simply to determine the collective properties of the mixture of species selected by the conditions imposed. In any case, a knowledge of the effects of various factors on microbial behavior is essential to understand or control the microbial activities in the defined process.

Among many factors affecting anaerobic microbial growth, the effects of pH and temperature are most commonly encountered and are most effective in selecting the microbial species that occupy a habitat.

1. Effect of Temperature

Of the physical factors affecting microbial growth in any environment, one of the most influential in the selection of species is temperature. Microorganisms possess no means of controlling internal temperature; therefore, the external temperature determines the

temperature within the cell. The maximum and minimum temperatures define the limit of the temperature range within which growth is possible; at lower or higher temperatures than these, no growth normally occurs. The optimum temperature is that at which growth is most rapid, i.e., at which the growth rate reaches its maximum value. It should be noted that the optimum temperature is based on the growth rate only and is not necessarily the temperature at which the maximum yield of cells occurs.

Generally, microbes that grow best at lower than 20°C are identified as psychrophiles, those that prefer temperatures higher than 45°C are classified as thermophiles, and those that grow best at temperatures between 20°C and 45°C are referred to as mesophiles. Typical optimum temperature ranges for the various species are shown in Table 1.

Microbial growth and survival are not necessarily affected in the same way by temperature. Temperatures above the maximum at which growth can occur are generally lethal and thus affect both growth and viability. Temperatures below the minimum at which growth is possible are not normally lethal and thus affect the growth but not viability. Among thermophilic microorganisms, those capable of growing at very high temperatures, i.e., above 60°C, are all procaryotes or archaeobacteria (Table 2).

The molecular basis of temperature limitation of growth is not well understood. Thermally induced changes in two classes of molecules, proteins and lipids, have been implicated in various mechanisms proposed to explain the effects of temperature on growth and viability.

Lipids containing fatty acids are essential structural components of membranes, both the cytoplasmic membrane, common to all cells, and the internal membranes of eucaryotes and some procaryotes. The melting points of such lipids increase with the degree of saturation of the fatty acids. The cytoplasmic membrane must maintain the proper balance of fluidity and structural integrity to allow control of the passage of molecules in and out of the cell while preventing the loss of essential cellular components. There is a direct correlation between the degree of saturation of the fatty acids of the membrane and the temperature range within which the organism is able to grow. An individual microorganism can also respond to growth at different temperatures within its tolerable range by changing the degree of saturation of its fatty acids. *Escherichia coli*, for example, can vary the percentages of saturated and unsaturated fatty acids in its cytoplasmic membrane by almost threefold when grown at 10 and 43°C, which are the extremes of its temperature range (Cherrington et al., 1990).

The effect of temperature on lipids is the basis of the proposed mechanisms for both cessation of growth at low temperatures and death at high temperatures. At low temperatures, the fluidity of the membrane may be decreased sufficiently to prevent the functioning of the transport systems, so that substrates cannot enter the cell rapidly enough to support even a low rate of growth. The transport of nutrients has been shown to vary with the growth temperature and degree of saturation of fatty acids in the membrane. At high temperature, membrane lipids may melt, causing loss of the structural integrity of the membrane and leakage of the cell contents.

Since microbial membranes are the sites of the majority of the essential life processes of the cells, any perturbation of the membrane might be expected to affect at least one of these processes. It has been suggested that the more complex internal membranes of the eucaryotes are more susceptible to loss of function with increasing temperature than is the cytoplasmic membrane. If so, this may explain the fact that eucaryotes have not been found at temperatures higher than 60^{o}C (see Table 1).

The present knowledge of membrane structure and function may not be sufficient to explain the differences in microorganisms capable of growing at the different temperature extremes. However, membrane function is undoubtedly important in determining the temperature at which an organism can grow or survive.

Most of the many different protein molecules in the cell function as enzymes that catalyze many reactions required for microbial growth. Proteins are also important components of cell structures such as ribosomes. For each of these roles, the specific protein involved must maintain a precise three-dimensional structure. Loss of function results from an alteration in the conformation of the molecule.

An increase in temperature affects proteins by causing thermal denaturation, an alteration of the functional spatial arrangement, which is usually irreversible. Thermal denaturation of proteins can cause loss of an essential enzyme function, alteration of membrane structure, or inactivation of the protein synthesizing mechanism due to alteration of ribosome conformation. Elevated temperatures thus can inactivate many essential processes in the cell by inactivating the protein involved. It has been proposed that growth ceases when the temperature reaches the point at which the most heat-sensitive essential protein of the cell is denatured.

In a mixed population of microorganisms, an increase of several degrees in the temperature may eliminate some species because their maximum limit has been exceeded. If

the species eliminated constituted a significant fraction of the total microbial population, other organisms whose growth rate had been increased by the temperature shift would be able to take advantage of the greater availability of nutrients and thus increase rapidly in numbers.

2. Effect of pH

A second environmental factor that influences the growth rate and limits growth is the hydrogen ion concentration, i.e., the acidity or alkalinity, of the aqueous environment. This is most conveniently expressed as pH, the negative logarithm of the molar concentration of the hydrogen ion (H^+).

The optimum pH value for any species is again that at which the growth rate is the most rapid. The minimum and maximum values that limit growth usually differ by three to four pH units . However, it must be recognized that a difference of four pH units represents a change in H^+ concentration of ten thousandfold, so that a narrow pH range actually involves a very broad range of H^+ concentrations. Some bacteria, a few protozoa, and many fungi are capable of growing over surprisingly broad pH ranges. One species of fungus, *Penicillium*, has been reported to have a minimum pH value for growth of 1.6 and a maximum of 11.1. The sulfur-oxidizing bacterium *Thiobacillus thiooxidans* grows most rapidly in the pH range 2.0 to 3.5 and has been reported to grow over the entire range of less than 0.5 to greater than 9.5.

The following discussions are based on two aspects of the relationship of microorganisms to pH. First, changes in the pH of the environment are most likely to be brought about by microorganisms themselves. Second, the internal pH of the cell, unlike the temperature, is not determined solely by the environment.

Many metabolic activities of microorganisms result in the formation of acidic or alkaline products. Acidogens convert substrates to a variety of products, many of which are organic acids. These are released from the cell and can result in a decrease in external pH of sufficient magnitude to inhibit growth. The lactic acid bacteria, for example, which are used in the manufacture of dairy products such as buttermilk and sour cream, convert the lactose in milk to lactic acid and lower the pH to approximately 4.0 to 4.5, at which point they cease to grow. If other, more acid-tolerant microorganisms are present, they may continue to grow, and the predominant species thus change due to the lowering of the pH by metabolic products. The acetic acid bacterium *Acetobacter* is used in the commercial production of

vinegar, in which ethanol is oxidized to acetic acid. These organisms can tolerate a pH value as low as 1.0.

Microorganisms also can increase the pH of their surroundings by the release of alkaline products or by the removal of certain ions from the environment. The most common cause of increase in pH is the metabolism of proteins, peptides, or amino acids. Deamination, removal of the amino group as ammonia, NH_3, results in the formation of ammonium hydroxide when the cell releases ammonia into the aqueous medium.

Since enzymes are active within only a specific and usually narrow pH range and display maximum activity at optimum pH, the effect of pH on enzymatic activity might explain the change of growth rate and viability due to the pH. Determinations of the pH optima of specific enzymes have shown that the enzymes of cells capable of growing in extremely acidic or alkaline conditions did not necessarily have pH optima corresponding to the optimum pH for growth of the cell, and they could even be totally inactive at a pH at which growth occurred. This could be a strong indication that internal pH of the cell was not identical with that of environment. A proposed mechanism for the transport of a variety of ions and organic compounds across the microbial cell membrane involves the establishment of a pH gradient across the membrane. The translocation of H^+ ions generates an electrochemical gradient, or proton-motive force, across the membrane, which drives the transport of other materials into the cell. The proton-motive force is also implicated in the energy-generating processes of cells that form ATP through an electron transport chain, and the external pH may affect the growth of microorganism by altering their ability to synthesize ATP.

The effects of pH on transport are both direct and indirect. A direct effect is exerted through alteration of the pH gradient across the membrane. For compounds that are transported by binding to specific membrane proteins, pH may control the configuration and thus the activity of the binding protein.

Although the mechanism by which pH affects growth rate and viability is not clearly known, it can be reasonably concluded that the effect of pH on the transport of materials across the membrane is a very important factor and perhaps the determining factor in influencing growth.

TABLE 1. Typical temperature ranges for various bacterial groups

Type	Temperature (°C)	
	Range	Optimum
Psychrophiles	-2 - 30	12 - 18
Mesophiles	20 - 45	30 - 40
Thermophiles	40 - 75	55 - 65

TABLE 2. Approximate upper temperature limits for different microorganisms

Organisms	Temperature (°C)
Protozoa	45 - 50
Eucaryotic algae	56
Fungi	60
Photosynthetic bacteria (including cyanobacteria)	70 - 73
Bacteria	99

Model Building and Use of a Model

A mechanistic model can be constructed only in situations where the investigator has sufficient knowledge of the mechanism to be able to deduce the functional relationship between input and output variables. However, there are many scientific areas, like many microbial processes, where basic knowledge of the phenomenon is insufficient to build a mechanistic model. Empirical models and statistical analysis, in this case, play an extremely important role in elucidating basic mechanisms in complex situations and thus providing better process control.

Model building should be an iterative process. Upon confronting the available data with the tentative model, which is based on subject-matter knowledge (i.e., microbiology, biochemistry, mathematics, and statistics), a researcher logically asks first whether there is any evidence that the model is inadequate. If so, the model should be modified or more data should be collected to check the model adequacy. If the model is adequate, the values of parameters along with their precision should be estimated, usually using the regression method in biological processes. If the precision is not high enough, additional data should be collected to repeat this procedure. Figure 1 illustrates the iterative process of building a model.

1. Response Surface Methodology

Response surface methodology (RSM) is a collection of mathematical and statistical techniques useful for analyzing problems where several independent variables influence a dependent variable or response, and the goal is to optimize this response. The independent variables are denoted as $x_1, x_2,....x_k$. It is assumed that these variables are continuous and controllable by the experimenter. The observed response, y, is assumed to be a random variable and may be written as:

$$y = f(x_1, x_2,....x_k) + \varepsilon \qquad \text{M-1}$$

where

y : observed response

x_i : variable i (i = 1, 2, ... k)

ε : random error

In most RSM problems, the form of the relationship between the response and the independent variables is unknown. Thus, the first step in RSM is to find a suitable approximation for the function (f). Usually, a low-order polynomial in some region of the independent variables is employed. If the response is well-modeled by a linear function of the independent variables, then the approximating function is a first-order model.

$$\hat{y} = \beta_o + \sum_{i=1}^{k} \beta_i x_i \qquad \text{M-2}$$

where

$\hat{y}$: model response
β_o : regression constant of the model
β_i : regression coefficients of the independent variables

If there is curvature in the system, then a polynomial of higher degree, such as a second-order model, must be used.

$$\hat{y} = \beta_o + \sum_{i=1}^{k} \beta_i x_i + \sum_{i=1}^{k} \beta_{ii} x_i^2 + \sum_{i} \sum_{\substack{j \\ i<j}} \beta_{ij} x_i x_j \qquad \text{M-3}$$

Almost all RSM problems utilize one or both of these approximating polynomials. A polynomial model is unlikely to be a reasonable approximation of f over the entire space of the independent variables, but it usually works well in a relatively small region.

RSM is a sequential procedure of collecting data, estimating polynomial coefficients, checking the adequacy of the model, modifying the model or the experimental design, and then conducting more experiments if necessary. Often, when we are at a point on the response surface that is remote from the optimum, there is little curvature in the system and the first-order model is appropriate (Eq. M-2). Once the region of the optimum has been found, a model of degree two or higher is usually required to approximate the response (Eq. M-3) because of the curvature in the surface, and an analysis is performed to locate the optimum, which is the set of independent variables such that the partial derivatives of the model response, $\hat{y}$, with respect to the individual independent variables equal to zero (Eq. M-4).

$$\frac{\partial \hat{y}}{x_1} = \frac{\partial \hat{y}}{x_2} = \ldots\ldots = \frac{\partial \hat{y}}{x_k} = 0 \qquad \text{M-4}$$

This optimum response is called the stationary point and it could represent a point of maximum response, a point of minimum response, or a saddle point. The eventual objective of the RSM is to determine the optimum operating conditions for the system, or to determine a region of the factor space in which the operating specifications are satisfied.

2. Experimental Design Techniques

The primary objective of designing experiments in most cases is to yield precise parameter estimates with a minimum of work and expense. The orthogonal design is a unique class of designs that minimize the variance of the regression coefficients. A first-order design is orthogonal if the off-diagonal elements of the product matrix of $X^{-1}X$, where X is the coefficient matrix, are all zero. This implies the cross-products of the columns of the X matrix sum to zero.

The class of orthogonal first-order designs includes the 2^k factorial and fractions of the 2^k series in which main effects do not interact with each other. This 2^k factorial design, however, does not afford an estimation of the experimental error unless some runs are repeated. A common method to include replication is to augment the design with several observations at the center. The estimation of experimental error will be discussed later. The addition of center points to the 2^k factorial design does not influence the coefficient estimation, but the estimate of the constant, β_o, becomes the overall average of all observations. Figure 2 represents the schematic diagram of a centered, first-order orthogonal design used to locate optimum conditions for a certain product formation rates with 3 variables: pH, temperature, and HRT.

An experimental design for fitting a second-order model must have at least three levels of each factor in order to estimate the model coefficients. The most widely used design for fitting a second-order model is the central composite design, which consists of a 2^k factorial design augmented by 2k axial points and some center points. One of the useful properties of this design is that it can be built up from the first-order design by adding the axial points and/or center points.

3. Validation of Model Adequacy

Accurate estimation of the parameter values is a key factor since the RSM and biokinetic study involve mathematical formulae with parameters to be evaluated. An observed value in most biochemical experiments may not be the true value or may not correspond to a model output due to an error caused by the instrument error, sampling imperfection, technician error, and other factors. The relationship between observations and model outputs are as follow:

$$y_i = \eta_i + \varepsilon_i \qquad \text{M-5}$$

where

y_i : an observed value at ith point
η_i : a model output at ith point
ε_i : a random error or disturbance at ith point
The error term is assumed to be independent and normally distributed having constant variance.

A model with independent variables, x, and parameters, θ, is to be fitted to experimental observations in order to estimate a set of parameters. Regardless of the form of the model, either linear or nonlinear, the least squares method provides unbiased estimates of the parameters with minimum variance. This is the best estimation method. For unbiased estimates, the parameter estimates converge to the true values as the number of observations increases.

Pictures or graphs are always a good starting point for examining lack of fit. Residuals, $\varepsilon_i = y_i - \eta_i$, can show inadequacies of the model. The first step is to plot the model outputs and the dependent variables against the independent variables. The next step is to plot the residuals against the predicted values and against each of the independent variables. This procedure is likely to give an indication of the following problems:

1. Outliers or erroneous observations.
2. Violation of the assumptions. For example, a model, $y = \beta_o + \beta_1 x + \varepsilon$, assumes a linear relationship between y and the dependent variable x, and independent, normally distributed errors with a constant variance.

The residual plots for the model and data set that have none of these problems are random variables and no patterns or trends exist. Existence of a certain pattern is evidence of

a weakness in the model. When a higher order model, for example, is more appropriate instead of the linear model, a residual plot with a curvature would be observed. A check of the constant variance assumption can be addressed in the plot of residuals versus an independent variable, x_i. Homogeneous error variances across values of x_i generates a random plot of residuals. If an increasing or decreasing pattern exists, it indicates the error variances increase or decrease with increasing values of x_i.

If the experiment is replicated at some settings, it is possible to conduct a test for lack of fit of the fitted model by partitioning residual sum of squares (RSS), ε_i^2 , into two parts: one for pure experimental error and the other for lack of fit. The relationship is shown as:

$$RSS = SSPEE + SSLOF \qquad \text{M-6}$$

where

RSS : residual sum of squares (sum of squares of error)

SSPEE : sum of squares due to pure experimental error

SSLOF : sum of squares due to lack of fit

Under the null hypothesis that the model is correct, independent estimates of the model error variance can be formed by dividing SSPEE and SSLOF by their respective degrees of freedom. These estimates are called mean squares (MS). An F-test between these two MSs for a specified value of α determines the significance of lack of fit.

If the model is correct and truly fits the data, the RSS consists only of pure measurement error. If the model does not fit, the RSS is inflated because it then consists of pure error plus error due to lack of fit.

What if there is no optimum point that you are looking for?.

You have many statistical options but the best methods are:

- Steepest ascent method
- Simplex method

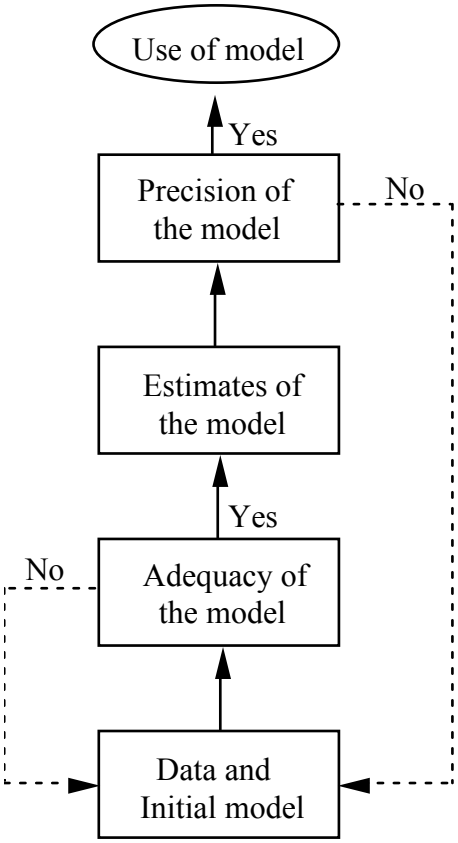


Figure 1--Schematic diagram of the model-building process.

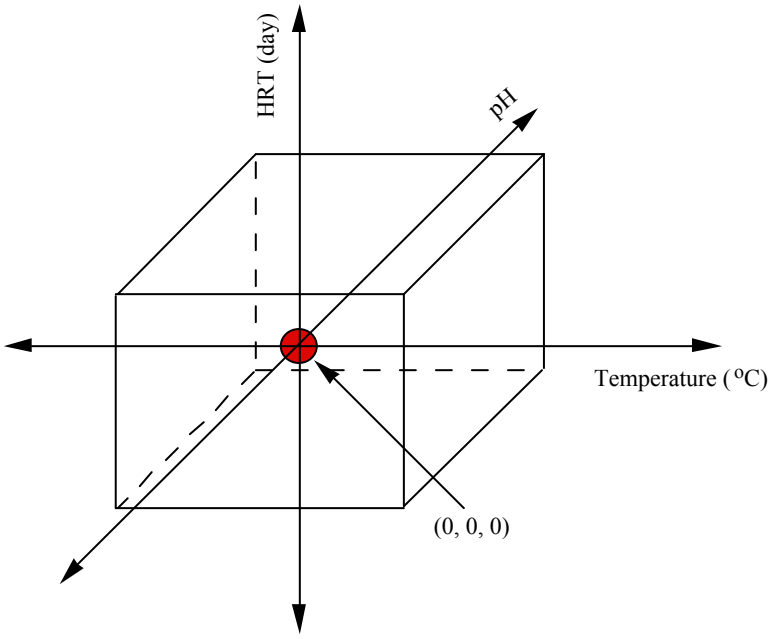


Figure 2--The schematic diagram of a first-order orthogonal design with 3 variables.

Appendix

THE BUG STORY.....

How Microorganisms Help to Treat Wastewater

The microscopic examination can be as useful a tool for the successful operation of the activated sludge system as other tests. Much can be learned about the state of the aeration basins based on the type of protozoa and metazoa present. Other helpful information is the description of the floc size and shape, and type and amount of filamentous bacteria present.

In a healthy mixed liquor sample, the microscopic examination should yield a predominance of stalked ciliates with moderate populations of other ciliates such as crawlers (creepers) or free swimmers. Other protozoa such as flagellates and amoebae should be found in small to scarce numbers. Although they are found in small populations, it is important that these other organisms be present with the ciliates in order to provide a more stable environment. For example, if an upset occurred that affected the higher life form, such as the D.O. dropping, a lower life form that can tolerate a low D.O. will survive.

The protozoa and metazoa have two important functions at a treatment plant. The first is to serve as indicator organisms to allow the viewer to determine the treatment level of the plant by microscopic evaluation. The second function is to clarify the effluent by feeding on the free bacterial cells in the solution that have not flocculated.

The Protozoa

Amoebae

The Amoebae are a group of very simple organisms classified by the presence of a pseudopodia or a "false foot". The pseudopodia are used by the amoebae for eating and moving. At the SRWTF we usually see two types of amoebae, shelled and naked. For our purposes the presence of either type has the same meaning. Amoebae usually predominate during a start up period when there is young sludge or after a major upset. They need large amounts of soluble food present in order to survive.

Flagellates

Flagellates are another group of primitive organisms, related to the amoebae. Flagellates get their name from the "tail like" projection called a flagella that extends from their bodies. The flagella is used for movement through the liquid. Like their relatives the amoebae, flagellates are present when there are large amounts of soluble food available (high F:M). They are found during start up when the sludge is young or after an upset, but will quickly predominate over the amoebae because they are more efficient feeders.

Ciliates

The Ciliates are more complex organisms than the amoebae and flagellates. They are classified as ciliates because of the tiny hairlike projections called cilia that are found on their bodies. In examination of an activated sludge sample the three important groups of ciliates are stalked, crawling and free-swimming.

Free-swimming ciliates

Free-swimming ciliates are identified by the cilia that surround most or all of their bodies. Free-swimmers swim faster than fiagellates so they can out compete them for food. Free-swimmers are usually found when no large flocs have been formed so that it is easier to swim around.

Crawling ciliates

Crawling ciliates have cilia mainly on the lower surface of their bodies that make them appear to be legs. In order for crawlers to dominate there must be large flocs present that impede the free-swimmers and flagellates movement and provide a surface for the crawlers to "walk" on. This means the F:M is low and the bacteria have started to flocculate as a survival response. Crawlers also require a high D.O. content in the mixed liquor.

Stalked ciliates

Stalked ciliates feed on the stray bacteria cells. They are easily identified from other ciliates because the main body of the organism is attached to a stalk that is usually implanted in the floc. Stalked ciliates are very efficient feeders and will predominate when the F:M is low. They also do best when there are high D.O. readings.

The Metazoa

Rotifers

Rotifers are very large as compared to other organisms. Unlike the protozoa, they are multicellular organisms. The rotifers are only found as the sludge age increases because it takes three days for their eggs to hatch, so if you waste 25% of your sludge a day you can lose 75% of the rotifer eggs.

Nematodes

Nematodes are also large in size as compared to the protozoa. Their eggs also require time to hatch and therefore they predominate at an older sludge age.

Filamentous Bacteria

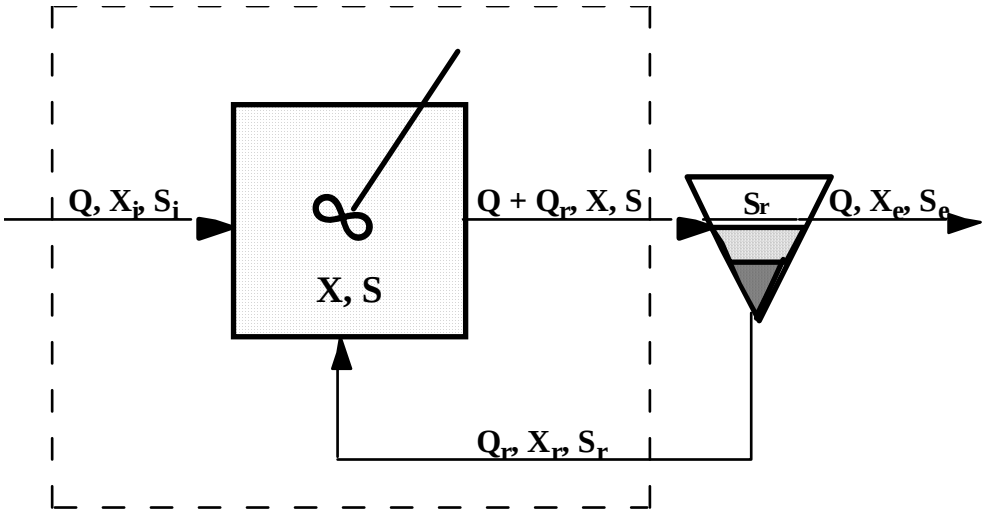
Filamentous bacteria serve as the backbone of floc formation. Sludge settles most efficiently when it contains a moderate number of filaments which provide structure for the floc and aid in the stripping of the water column. The floc cannot form properly if there are too few filaments, and the floc cannot settle properly if there are too many. The filamentous bacteria are analyzed in two ways: their effect on floc structure and their abundance.

Uhmm....

Since energy is required to maintain the integrity of the MO, the MO will die if at least enough energy for maintenance is not supplied. Many microbes can store reserves of carbon and energy sources (e.g., poly-b-hydroxybuterate, starch, oils) and some nutrients (e.g., polyphosphates) for use during periods of starvation. These reserves may not supply all of the resources needed to maintain the cell and/or they will eventually be consumed. Other cell constituents such as enzymes and nucleic acids may then be utilized to provide maintenance energy. At some point, the cell becomes **"moribund"** (bound to death) because too much of some necessary component has been degraded. If certain kinds of growth substrate become available after this point, the population of starved organisms may actually become non-viable (unable to cultured, dead) at a higher rate. Apparently, this occurs because the cell attempts to synthesize transport proteins or enzymes that will allow it to assimilate and metabolize the substrate, misallocates critical cell resources, and in so doing actually accelerates its own death. This phenomenon is called **"substrate accelerated death."**

Biokientic Evaluations

System with recycle



MATERIAL BALANCE (around the dashed line only)

Biomass (X): Net change = Input - Output + Growth - Decay

$$\left(\frac{dX}{dt}\right)_{net}V = (QX_i + Q_rX_r) - (Q + Q_r)X + \left\{\left(\frac{dX}{dt}\right)_g - \left(\frac{dX}{dt}\right)_d\right\}V$$

$$\left(\frac{dX}{dt}\right)_{net} = \frac{(QX_i + Q_rX_r) - (Q + Q_r)X}{V} + (\mu - k_d)X$$

$$= \frac{1}{V}\{Q(X_i - X) + Q_r(X_r - X)\} + (\mu - k_d)X \qquad \therefore \ \mu = \frac{\mu_m S}{K_s + S}$$

Substrate (S): Net change = Input - Output + Utilization

$$\left(\frac{dS}{dt}\right)_{net}V = (QS_i + Q_rS_r) - (Q + Q_r)S + \left(\frac{dS}{dt}\right)_u V$$

$$\left(\frac{dS}{dt}\right)_{net} = \frac{(QS_i + Q_rS_r) - (Q + Q_r)S}{V} + \left(\frac{dS}{dt}\right)_u$$

$$= \frac{1}{V}\{Q(S_i - S) + Q_r(S_r - S)\} + \left(\frac{dS}{dt}\right)_u$$ Here $S_r = S$, see assumptions

$$= \frac{Q}{V}(S_i - S) + \left(\frac{dS}{dt}\right)_u \qquad \therefore \left(\frac{dS}{dt}\right)_u = -\frac{1}{Y}\left(\frac{dX}{dt}\right)_g = -\frac{\mu_m}{Y}\frac{SX}{K_s + S}$$

No substrate utilization during endogenous decay

ASSUMPTIONS:

(1) No wasting of sludge

(2) No biological reaction in the settler (we don't consider the settler volume);

$[S] = [S_r] = [S_e]$

(3) If there is no recycle;

$[X]=[X_e],\ \tau = \frac{V}{Q}$

otherwise

$[X] \neq [X_e],\ \tau = \frac{V}{Q + Q_r}$

BATCH SYSTEM

* No inflow & outflow

* Only equilibrium exists - No steady-state condition

Biomass (X): Net change = Growth - Decay

$$\left(\frac{dX}{dt}\right)_{net} V = \left\{\left(\frac{dX}{dt}\right)_g - \left(\frac{dX}{dt}\right)_d\right\} V$$

$$\left(\frac{dX}{dt}\right)_{net} = (\mu - k_d)X = \left(\frac{\mu_m S}{K_s + S} - k_d\right)X$$

(i) Euler method

$$\frac{X_{t+1} - X_t}{\Delta t} = \left(\frac{\mu_m S_t}{K_s + S_t} - k_d\right)X_t$$

$$X_{t+1} = \left\{1 + \left(\frac{\mu_m S_t}{K_s + S_t} - k_d\right)\Delta t\right\} X_t$$

(ii) Direct solution

$$\int_{X_o}^{X} \frac{dX}{X} = (\mu - k_d) \int_0^t dt$$

$$\ln \frac{X}{X_o} = (\mu - k_d)t$$

$$X = X_o e^{(\mu - k_d)t} \quad \text{where } \mu = \frac{\mu_m S}{K_s + S}$$

Substrate (S): Net change = Substrate utilization

$$\left(\frac{dS}{dt}\right)_{net} V = \left(\frac{dS}{dt}\right)_u V$$

$$\left(\frac{dS}{dt}\right)_u = -\frac{1}{Y}\left(\frac{dX}{dt}\right)_g = -\frac{\mu_m}{Y}\frac{SX}{K_s + S}$$

No substrate utilization during endogenous decay

(i) Euler method

$$\frac{S_{t+1} - S_t}{\Delta t} = - \frac{\mu_m}{Y} \frac{S_t X_t}{K_s + S_t}$$

$$S_{t+1} = \left(1 - \frac{\mu_m}{Y} \frac{X_t}{K_s + S_t} \Delta t\right) S_t$$

(ii) Direct solution

$$\left(\frac{dS}{dt}\right)_u = \left(- \frac{\mu_m X}{Y}\right) \frac{S}{K_s + S}$$

$$\int_{S_o}^{S} \frac{Ks + S}{S} dS = - \frac{\mu_m X}{Y} \int_0^t dt$$

$$K_s \ln \frac{S}{S_o} + S - S_o = \frac{\mu_m X}{Y} t \quad \rightarrow \quad K_s \ln \frac{S_o}{S} + S_o - S = \frac{\mu_m X}{Y} t$$

$$\ln \frac{S_o}{S} = - \frac{S_o - S}{K_s} + \frac{\mu_m X}{Y} t \quad \rightarrow \quad \text{Dependent variables can't be separated !!!}$$

then,

$$\frac{\ln \frac{S_o}{S}}{S_o - S} = \frac{\mu_m}{Y K_s} \frac{X}{S_o - S} t - \frac{1}{K_s} \quad \rightarrow \quad \text{Linear solution !!!}$$

Calculation Procedure (Euler method):

(1) Set equation(s) & link X_{t+1} & S_{t+1}
(2) Set parameters, independent, & dependent variables
(3) Guess initial parameter values (mostly μ_m, K_s, Y, k_d)
(4) X_o = initial [X] & S_o = initial [S], when $t = 0$
(5) Get calculated [X] & [S]
(6) Compare calculated values with experimental results
(7) Keep changing initial parameter values to give the best curve fittings

We can get the **pattern of microbial growth and substrate utilization** with time, if all parameter values are known.

Calculation Procedure (Direct solution):

(1) Set proper equation(s) to describe the rxn

(2) Make necessary program(s) describing the rxn

(3) Apply proper statistics at this point if necessary or (7)

(4) Set parameters, independent, & dependent variables

(5) Guess initial parameter values

(6) Iterate program with **raw data** to give the best curve fittings

(7) Necessary statistical test

Get K_s, μ_m, k_d first from the previous Eq. & here slope = $\frac{\mu_m}{YK_s}$ and Y-intercept = - $\frac{1}{K_s}$

Compare K_s value with the previous one and if it is statistically same, we can get Y value from the slope with the previous μ_m value.

CONTINUOUS SYSTEM (CSTR)

(A) STEADY STATE SOLUTION

* Use data **only** taken at **steady-state**

Biomass (X) : See material balance

$$\left(\frac{dX}{dt}\right)_{net} = \frac{1}{V}\{Q(X_i - X) + Q_r(X_r - X)\} + (\mu - k_d)X$$

at steady state, $\left(\frac{dX}{dt}\right)_{net} = 0$ & divided by X

$$\mu - k_d = \frac{1}{VX}\{Q(X - X_i) + Q_r(X - X_r)\} \qquad \mu = \frac{\mu_m S}{K_s + S} = Y\frac{Q(S_i - S)}{VX}$$

$$\therefore S = \frac{K_s\mu_n}{\mu_m - \mu_n}$$ **S : (1) Actual [S] to get the parameter values**

(2) Calculated [S] to get final [S], [X]

(1) if $X_i \neq 0$, w/ recycle $\qquad \tau = \frac{V}{Q + Q_r}$

$$\mu_1 = \frac{Q}{V}\left(1 - \frac{X_i}{X}\right) + \frac{Q_r}{V}\left(1 - \frac{X_r}{X}\right) + k_d$$

(2) if $X_i \neq 0$, w/o recycle $\qquad \tau = \frac{V}{Q}$

$$\mu_2 = \frac{1}{\tau}\left(1 - \frac{X_i}{X}\right) + k_d$$

(3) if $X_i = 0$, w/ recycle $\qquad \tau = \frac{V}{Q + Q_r}$

$$\mu_3 = \frac{Q}{V} + \frac{Q_r}{V}\left(1 - \frac{X_r}{X}\right) + k_d$$

(4) if $X_i = 0$, w/o recycle $\qquad \tau = \frac{V}{Q}$

$$\mu_4 = \frac{V}{Q} + k_d = \frac{1}{\tau} + k_d \qquad \frac{1}{\tau} = \mu - k_d$$

Substrate (S) : See material balance

$$\left(\frac{dS}{dt}\right)_{net} = \frac{Q}{V}(S_i - S) + \left(\frac{dS}{dt}\right)_u$$

at steady state $\left(\frac{dS}{dt}\right)_{net} = 0$

$$-\left(\frac{dS}{dt}\right)_u = \frac{Q}{V}(S_i - S) \qquad \therefore \left(\frac{dS}{dt}\right)_u = -\frac{1}{Y}\left(\frac{dX}{dt}\right)_g = -\frac{\mu_m}{Y}\left(\frac{XS}{K_s + S}\right)$$

$$\frac{\mu_m S}{K_s + S} X = \frac{YQ(S_i - S)}{V} = \mu X \qquad \therefore \mu = Y\frac{Q(S_i - S)}{VX}$$

Put μ into this Eq. & arrange for X;

$$\left[\frac{1}{VX}\{Q(X - X_i) + Q_r(X - X_r)\} + k_d\right]X = \frac{YQ(S_i - S)}{V}$$

$$\left(\frac{Q + Q_r}{V} + k_d\right)X = \frac{YQ(S_i - S) + (QX_i + Q_r X_r)}{V}$$

(1') if $X_i \neq 0$, w/ recycle $\tau = \dfrac{V}{Q+Q_r}$

$$X = \frac{YQ(S_i - \boldsymbol{S'}) + (QX_i + Q_rX_r)}{Q + Q_r + k_dV}$$

(2') if $X_i \neq 0$, w/o recycle $\tau = \dfrac{V}{Q}$

$$X = \frac{YQ(S_i - \boldsymbol{S'}) + QX_i}{Q + k_dV}$$

(3') if $X_i = 0$, w/ recycle $\tau = \dfrac{V}{Q+Q_r}$

$$X = \frac{YQ(S_i - \boldsymbol{S'}) + Q_rX_r}{Q + Q_r + k_dV}$$

(4') if $X_i = 0$, w/o recycle $\tau = \dfrac{V}{Q}$

$$X = \frac{YQ(S_i - \boldsymbol{S'})}{Q + k_dV} = \frac{Y(S_i - \boldsymbol{S'})}{1 + k_dt}$$

***S'*: (1) Actual [*S*] to get the parameter values (i.e *S* =*S'*)**

(2) Guessed [*S*] to get final [*S*], [*X*] (i.e *S* ≠ *S'*)

CASE I

∗ **To get the parameter values** from experimental data

∗ Nonlinear & Linear estimation

∗ Direct solution & Iterative process

(A) Nonlinear Parameter Estimation (In most case w/ computer)

Calculation Procedure:

(1) Set proper equation(s) to describe the rxn
(2) Make necessary program(s) describing the rxn
(3) Apply proper statistics at this point if necessary or (7)
(4) Set parameters, independent, & dependent variables
(5) Guess initial parameter values
(6) Iterate program with **raw data** to give the best curve fittings
(7) Necessary statistical test

(ex) If $X_i \neq 0$ w/ recycle

(1)-(3)

$$S = \frac{K_s \mu_1}{\mu_m - \mu_1} \qquad \mu_1 = \frac{Q}{V}\left(1 - \frac{X_i}{X}\right) + \frac{Q_r}{V}\left(1 - \frac{X_r}{X}\right) + k_d$$

$$X = \frac{YQ(S_i - \boldsymbol{S'}) + (QX_i + Q_r X_r)}{Q + Q_r + k_d V} \qquad \boldsymbol{S'} = S = \text{Actual } [S]$$

(4) parameters : K_s, μ_m, Y, k_d

independent variables : Q, S_i, Q_r, V, X_i

dependent variables : S, X, (X_r)

(5) Guess initial parameter values based on the literature

(6) Get the parameter values to give the best curve fitting (iterative)

(B) Linear Parameter Estimation (in limited case)

Calculation Procedure:

(1) Set linear equation(s) by necessary transformations & assumptions

(2) Set parameters, independent, & dependent variables

(3) Get the straight line(s) from the transformed data

(4) Get the parameter values from the slope, Y or X-intercepts

(5) Necessary statistical test

(ex) If $X_i = 0$ w/o recycle

(1) $\mu = \dfrac{\mu_m S}{K_s + S} = Y\dfrac{Q\,(S_i - S)}{VX}$ from definitions

$\dfrac{1}{\tau} = \mu - k_d$ from Eq. (4')

$\dfrac{VX}{Q\,(S_i - S)} = \dfrac{YK_s}{\mu_m}\dfrac{1}{\boldsymbol{S'}} + \dfrac{Y}{\mu_m} = \dfrac{K_s}{k}\dfrac{1}{\boldsymbol{S'}} + \dfrac{1}{k}$ $\mu_m = Yk$

and

$\dfrac{1}{\tau} = Y\dfrac{Q(S_i - \boldsymbol{S'})}{VX} - k_d = Y\dfrac{(S_i - \boldsymbol{S'})}{\tau X} - k_d$

or

$\tau = \dfrac{Y}{k_d}\dfrac{(S_i - \boldsymbol{S'})}{X} - \dfrac{1}{k_d}$ from Eq. (4') $\boldsymbol{S'} = S$ =Actual [S]

(2) parameters : K_s, μ_m, Y, k_d

independent variables : τ, S_i

dependent variables : S, X

(3)-(4)

Slope : $\dfrac{YK_s}{\mu_m}$ or $\dfrac{K_s}{k}$ or Y or $\dfrac{Y}{k_d}$

Y intercept : $\dfrac{Y}{\mu_m}$ or $\dfrac{1}{k}$ or $-k_d$ or $-\dfrac{1}{k_d}$

∗ When X_i=0, w/o recycle

From Eq. (4); $\mu = \dfrac{1}{\tau} + k_d = \dfrac{1 + k_d\tau}{\tau} = \dfrac{\mu_m S}{K_s + S}$

$\mu_m S\tau = (1+k_d\tau)(K_s + S)$

$(\mu_m\tau - k_d\tau - 1)S = K_s(1 + k_d\tau)$

$$S = \frac{K_s(1+k_d\tau)}{(\mu_m - k_d)\tau - 1}$$

From Eq. (4'); $X = \dfrac{Y(S_i - \boldsymbol{S'})}{1+k_d\tau}$ $\rightarrow$ $1 + k_d\tau = \dfrac{Y(S_i - \boldsymbol{S'})}{X}$ here, $S = \boldsymbol{S'}$

so,

$$\frac{\mu_m S}{K_s + S} = \frac{Y(S_i - S)}{X\tau}$$

$X\tau\mu_m S = Y(S_i - S)(K_s + S)$

$X = \dfrac{Y(S_i - S)(K_s + S)}{\tau\mu_m S}$ $\rightarrow$ direct solution w/ computer

$S \rightarrow S^2 + (K_s - S_i + X\tau\dfrac{\mu_m}{Y})S - S_iK_s = 0$ $\rightarrow$ quadratic equation

$S = \dfrac{K_s(1+k_d\tau)}{(\mu_m - k_d)\tau - 1}$ or $S^2 + (K_s - S_i + X\tau\dfrac{\mu_m}{Y})S - S_iK_s = 0$
$X = \dfrac{Y(S_i - S)}{1+k_d\tau}$ or $X = \dfrac{Y(S_i - S)(K_s + S)}{\tau\mu_m S}$

CASE II

* **To predict final [*X*], [*S*] at steady state** with known biokinetic coefficients

* Must guess either [*S*] or [*X*], here I guess [*S*′]

* Trial & Error method

Calculation Procedure:

(1) Set proper equation(s)
(2) Put guessed value (***S*′**) into the equation & get [*X*]
(3) Get calculated [*S*]
(4) Compare [*S*] with ***S*′**
(5) Keep changing ***S*′** until *S* = ***S*′**

In spread sheet:

***S*′**	*X*	μ	*S*

(B) NONSTEADY STATE SOLUTION

* Use whole data to get parameter values from experiment **at each HRT**

* To predict the change in [*X*], [*S*] with time **at each run** with known parameter values

* We normally get information for **1 run of experiment** (ex: only at 1 d HRT)

Biomass (X) : See material balance

$$\left(\frac{dX}{dt}\right)_{net} = \frac{1}{V}\{Q(X_i - X) + Q_r(X_r - X)\} + (\mu - k_d)X$$

(i) Euler method:

$$\frac{X_{t+1} - X_t}{\Delta t} = \frac{1}{V}\{Q_t(X_{it} - X_t) + Q_{rt}(X_{rt} - X_t)\} + \left(\frac{\mu_m S_t}{K_s + S_t} - k_d\right)X_t$$

(1) if $X_i \neq 0$, w/ recycle $\tau = \dfrac{V}{Q + Q_r}$

$$X_{t+1} = X_t + \left[\frac{1}{V}\{Q_t(X_{it} - X_t) + Q_{rt}(X_{rt} - X_t)\} + \left(\frac{\mu_m S_t}{K_s + S_t} - k_d\right)X_t\right]\Delta t$$

(2) if $X_i \neq 0$, w/o recycle $\tau = \dfrac{V}{Q}$

$$X_{t+1} = X_t + \left\{\frac{1}{\tau_t}(X_{it} - X_t) + \left(\frac{\mu_m S_t}{K_s + S_t} - k_d\right)X_t\right\}\Delta t$$

(3) if $X_i = 0$, w/ recycle $\tau = \dfrac{V}{Q + Q_r}$

$$X_{t+1} = X_t + \left[\frac{1}{V}\{Q_{rt}(X_{rt} - X_t) - Q_t X_t\} + \left(\frac{\mu_m S_t}{K_s + S_t} - k_d\right)X_t\right]\Delta t$$

(4) if $X_i = 0$, w/o recycle $\tau = \dfrac{V}{Q}$

$$X_{t+1} = X_t + \left(\frac{\mu_m S_t}{K_s + S_t} - k_d - \frac{1}{\tau_t}\right)X_t\Delta t$$

(ii) Direct solution:

$a = \mu$, $b = k_d$, $c = \dfrac{Q + Q_r}{V}$, $A = a - b - c$ $B = \dfrac{QX_i + Q_r X_r}{V}$

$$\left(\frac{dX}{dt}\right)_{net} = \left(\mu - k_d - \frac{Q + Q_r}{V}\right)X + \frac{QX_i + Q_r X_r}{V}$$

$$= AX + B$$

$$\int_{X_o}^{X} \frac{dX}{AX + B} = \int_0^t dt$$

$$\frac{1}{A}\ln\left(\frac{AX + B}{AX_o + B}\right) = t$$

(1) if $X_i \neq 0$, w/ recycle $\tau = \dfrac{V}{Q+Q_r}$

$$X = \left(X_o + \frac{\dfrac{QX_i + Q_r X_r}{V}}{\mu - k_d - \dfrac{1}{\tau}} \right) e^{(\mu - k_d - \tau^{-1})t} - \frac{\dfrac{QX_i + Q_r X_r}{V}}{\mu - k_d - \dfrac{1}{\tau}}$$

(2) if $X_i \neq 0$, w/o recycle $\tau = \dfrac{V}{Q}$

$$X = \left(X_o + \frac{X_i}{\mu\tau - k_d\tau - 1} \right) e^{(\mu - k_d - \tau^{-1})t} - \frac{X_i}{\mu\tau - k_d\tau - 1}$$

(3) if $X_i = 0$, w/ recycle $\tau = \dfrac{V}{Q+Q_r}$

$$X = \left(X_o + \frac{Q_r X_r}{\mu V - k_d V - Q - Q_r} \right) e^{(\mu - k_d - \tau^{-1})t} - \frac{Q_r X_r}{\mu V - k_d V - Q - Q_r}$$

(4) if $X_i = 0$, w/o recycle $\tau = \dfrac{V}{Q}$

$$X = X_o e^{(\mu - k_d - \tau^{-1})t}$$

$$AX = (AX_o + B)e^{At} - B \qquad X = \left(X_o + \frac{B}{A} \right) e^{At} - \frac{B}{A}$$

$$X = \left(X_o + \frac{B}{a - b - c} \right) e^{(a - b - c)t} - \frac{B}{a - b - c}$$

Substrate (S) : See material balance

$$\left(\frac{dS}{dt} \right)_{net} = \frac{Q}{V}(S_i - S) - \frac{\mu_m}{Y}\left(\frac{SX}{K_s + S} \right)$$

(i) Euler method

$$\frac{S_{t+1} - S_t}{\Delta t} = \frac{Q_t}{V}(S_{it} - S_t) - \frac{\mu_m}{Y}\left(\frac{S_t X_t}{K_s + S_t} \right)$$

Link proper Eq. for X (i.e. $X_i \neq 0$ or $X_i = 0$)

$$S_{t+1} = S_t + \left\{ \frac{Q_t}{V}(S_{it} - S_t) \; - \; \frac{\mu_m}{Y}\left(\frac{S_t X_t}{K_s + S_t}\right) \right\} \Delta t$$

(ii) Direct solution

$$\left(\frac{dS}{dt}\right)_{net} = \frac{Q}{V}(S_i - S) - \frac{\mu_m}{Y}\left(\frac{SX}{K_s + S}\right)$$

$$\left(\frac{dS}{dt}\right)_{net} = \frac{-S^2 + \left(S_i - K_s - \frac{\mu_m}{Y}\frac{V}{Q}X\right)S + S_i K_s}{\frac{V}{Q}(K_s + S)}$$

Dependent variables can't be separated !!!

Calculation Procedure (Euler method):

(1) Set equations & link them together
(2) Set parameters, independent, & dependent variables
(3) Guess initial parameter values (mostly μ_m, K_s, Y, k_d)
(4) X_o = initial [X], $X_{io} = 0$, $S_o = S_{io}$ = initial [S_i], when $t = 0$
(5) Get calculated [X] & [S]
(6) Compare calculated values with experimental results
(7) Keep changing initial parameter values to give the best curve fittings

We can get the **pattern of microbial growth and substrate utilization** with time, if all parameter values are known

Calculation Procedure (Direct solution):

(1) Set proper equation(s) to describe the rxn.
(2) Make necessary program(s) describing the rxn.
(3) Apply proper statistics at this point if necessary or (7)
(4) Set parameters, independent, & dependent variables
(5) Guess initial parameter values
(6) Iterate program with **raw data** to give the best curve fittings

(7) Necessary statistical test

(I) Reaction order determination (nonlinear least squares method)

$r_s = kS^a$

r = reaction rate

k = reaction rate constant

S = number of organisms

a = reaction order

$$\frac{dS}{dt} = kS^a$$

$$\int_{S_o}^{S} \frac{dS}{S^a} = \int_0^t dt$$

$$\frac{1}{1-a}(S^{1-a} - S_o^{1-a}) = kt$$

$$S^{1-a} = (1-a)kt + S_o^{1-a}$$

$$S = \left((1-a)kt + S_o^{1-a}\right)^{1/1-a} \qquad (a \neq 1)$$

(II) CSTRs in series (steady state, 1st order reaction)

$$\tau_1 = \frac{S_i - S_1}{r_{s1}} = \frac{S_i - S_1}{kS_1} \qquad S_1 = \frac{S_i}{k\tau_1 + 1}$$

$$\tau_2 = \frac{S_i - S_2}{r_{s2}} = \frac{S_i - S_2}{kS_2} \qquad S_2 = \frac{S_1}{k\tau_2 + 1} = \frac{S_i}{(k\tau_1 + 1)(k\tau_2 + 1)}$$

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$$\tau_n = \frac{S_{n-1} - S_n}{r_{sn}} = \frac{S_{n-1} - S_n}{kS_n} \qquad S_n = \frac{S_{n-1}}{k\tau_n + 1} = \frac{S_i}{(k\tau + 1)^n}$$

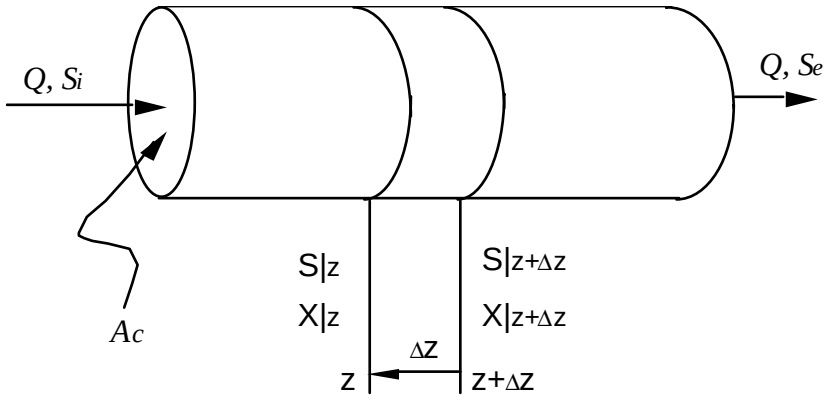
$(\tau_1 = \tau_2 = \cdots\cdots = \tau_n)$

$$k\tau + 1 = \left(\frac{S_i}{S_n}\right)^{1/n}$$

$$\tau = \frac{\left(\frac{S_i}{S_n}\right)^{1/n} - 1}{k} = \frac{V}{Q}$$

if $n = 1$, it will be same as single CSTR

(III) PFR (Diffusion in z-direction only, steady state)



Q : flow rate

S_i : substrate concentration at the reactor entrance

S_e : substrate concentration at the reactor exit

A_c : cross sectional area

z : the distance from the reactor entrance

Δz : the length of infinitesimal reactor volume

$S|_z$: the substrate concentration at the distance z

$S|_{z+\Delta z}$: the substrate concentration at the distance z+Δz

$X|_z$: the biomass concentration at the distance z

$X|_{z+\Delta z}$: the biomass concentration at the distance z+Δz

Overall mass balance around the substrate:

Net accumulation = convection + diffusion/dispersion + reaction

$$(S|_{t+\Delta t} - S|_t)\, A_c\, \Delta z = Q(S|_z - S|_{z+\Delta z})\, \Delta t + A_c\, (J_z|_z - J_z|_{z+\Delta z})\, \Delta t + r\, A_c\, \Delta z\, \Delta t$$

Simplifying by dividing by $A_c\, \Delta z\, \Delta t$

$$\frac{S|_{t+\Delta t} - S|_t}{\Delta t} = \frac{Q}{A_c}\,\frac{S|_z - S|_{z+\Delta z}}{\Delta z} + \frac{J_z|_z - J_z|_{z+\Delta z}}{\Delta z} + r$$

$$\frac{\Delta S}{\Delta t} = - v_z \frac{\Delta S}{\Delta z} - \frac{\Delta J_z}{\Delta z} + r$$

Taking limit as Δt and Δz approach to zero

$$\frac{\partial S}{\partial t} = - v_z \frac{\partial S}{\partial z} - \frac{\partial J_z}{\partial z} + r$$

Fick's first law $J_z = - D \frac{\partial S}{\partial z}$

J_z = flux in z direction (mass/area/time)

D = diffusivity (area/time)

$$\frac{\partial S}{\partial t} = D \frac{\partial^2 S}{\partial z^2} - v_z \frac{\partial S}{\partial z} + r$$

Assuming no diffusion in biomass

$$Q\,(S|_z - S|_{z+\Delta z}) - r_s A_c\,\Delta z = \frac{\partial S}{\partial z} A_c\,\Delta z$$

$$\frac{Q\,(S|_z - S|_{z+\Delta z})}{\Delta z} - r_s A_c = \frac{\partial S}{\partial z} A_c$$

if $\Delta z \rightarrow 0$

$$Q \frac{\partial S}{\partial z} - r_s A_c = \frac{\partial S}{\partial z} A_c$$

at steady state, $\frac{\partial S}{\partial z} = 0$

$$\frac{dS}{dz} = \frac{A_c}{Q} r_s \qquad (r_s = kS)$$

$$\frac{1}{k} \int_{S_i}^{S} \frac{dS}{S} = \frac{A_o}{Q} \int_0^z dz$$

$$\frac{1}{k} \ln \frac{S_i}{S} = \frac{A_c D}{Q} = \frac{V}{Q} = \tau$$