

## **Biological Treatment Processes Class Notes.**

These notes are prepared for my use as a teaching aid in the class. They contain the minimal material that I want to discuss. I may elaborate past the contents of these notes. Students should still be prepared to make their own notes in addition to these and read the applicable sections in the text book.

### **Chapter 1. Overview of Wastewater Engineering**

Simply defined, wastewater is water that has picked up various contaminants during its use in domestic, commercial or industrial applications. Not only does the scientific community realize that these components must be removed before the water can be put back into a natural water body, but the government regulates this more strictly every year.

Table 1-3 (overhead) presents some minimal national standards for removal of some of the major constituents in wastewater. Our improved ability to detect chemicals and bacteria in water and environmental samples and newer studies demonstrating unexpected health and environmental effects of components of wastewater continue to challenge the wastewater treatment industry.

#### **Some Terminology**

- Primary treatment processes - the physical settling of insoluble solids from a wastewater stream. Usually called primary settling.
- Secondary treatment - the biological treatment system
- Secondary clarification - the separation of biological solids from the main wastewater stream
- Tertiary treatment - Any treatment following secondary treatment, usually for nutrient removal.

Table 1-4 (overhead) lists some basic wastewater treatment levels and their capabilities. Table 1-5 lists some typical contaminants and which processes can be used to remove them.

The rest of the chapter is an introduction to topics that we will cover in more detail later. Read the chapter for your own information.

#### **Some Quick information for future reference**

Due to the nature of the wastewater treatment business we will use both American and metric units in this class. You will have to do a lot of converting. For example, concentrations are most often expressed in mg/L (or  $\mu\text{g/L}$ ) but flows are expressed in MGD (million gallons/day). You must be able to convert back and forth.

To make some conversions easier for you here are some conversion factors for units

1 cubic foot = 7.481 gallons  
1 gallon = 3.785 liters  
1 m<sup>3</sup> = 1000 liters = 264.2 gallons This will be used most often.

Conversion of mg/L to lb/Mgal multiply by 8.34  
Other conversions found in Appendix A of text

Molarity (M): number of mol of solute per liter of solution  
e.g. ethanol C<sub>2</sub>H<sub>5</sub>OH (46 g/mol); use 23 g/L = 0.5 M

## Chapter 2. Constituents in Wastewater

Table 2-2 (overhead) lists some typical constituents of concern in wastewater. In this class we will mostly deal with those characteristics that are important to biological treatment processes.

### ***Physical Characteristics***

#### **Solids**

All contaminants in water contribute to the solids load of that water. Solids can be divided into several categories. Table 2-4 (overhead) presents a list of definitions of the different types of solids found in wastewater. Figure 2-3 (overhead) presents the interrelationships of the various categories and can be used as a learning aid for problem solving when the method information is added to the figure.

#### **Measurement (Figure 2-3, Overhead)**

##### i) Total solids (TS)

- |        |   |
|--------|---|
| Method | a. Weigh a porcelain or aluminum dish   |
|        | b. Add a specific volume of well mixed sample                                     |
|        | c. Put in oven at 104°C for 4 hr (till constant wt.)<br>(removes water)           |
|        | d. Weigh again, after cooling, difference of this and wt. from a. is total solids |

##### ii) Suspended solids (SS)

- |        |  |
|--------|--|
| Method | a. Weigh glass fiber filter paper  |
|        | b. Filter specific volume of well mixed sample<br>solids will be retained on filter.   |
|        | c. Put in oven at 104°C for 4 hr (till constant wt.)                                   |
|        | d. Weigh again, after cooling, difference of this and wt. from a. is suspended solids. |

##### iii) Filterable solids (FS)

- |        |   |
|--------|---|
| Method | a. Take filtrate from SS put in pre weighed dish. |
|--------|---|

- b. Put in oven at 104°C for 4 hr (till constant wt.).
- d. Weigh again, after cooling, difference of this and wt. of dish is filterable solids.

iv) Volatile solids (VS) and Fixed solids (FS)

- Method
- a. Take residue from SS or FS and put in oven at 550°C for 4 h. (till constant weight).
  - b. cool and weigh.
  - c. the weight lost during the process is the volatile solids  
 VSS = volatile suspended solids = organic portion  
 VFS = volatile filterable solids = inorganic portion

The volatile suspended solids (VSS) are often considered to be the most important as they comprise the bulk of the insoluble organic compounds present in water. This fraction also contains the bacterial content of the sample. The volatile filterable solids (VFS) are the dissolved organic compounds which may contain toxic, carcinogenic and or other problem causing organic compounds.

Example 2-4 goes through one set of solids determination calculations. For variety, I have my own example.

Determine suspended solids for a water sample

volume filtered = 200 mL  
 filter mass = 1.3255 g  
 filter after drying 1.3286 g

suspended solids = (filter + solids) - filter  
 $1.3286 \text{ g} - 1.3255 \text{ g} = .0031 \text{ g}$   
 this was in 200 mL  
 $0.0031 \text{ g} / 200 \text{ mL} \times 1000 \text{ mg/g} \times 1000 \text{ mL/L}$   
 $= 15.5 \text{ mg/L}$

additional to this - put filter in muffle furnace at 550°C for 4 h.

new wt. of filter = 1.3274 g

Determine VSS and FSS

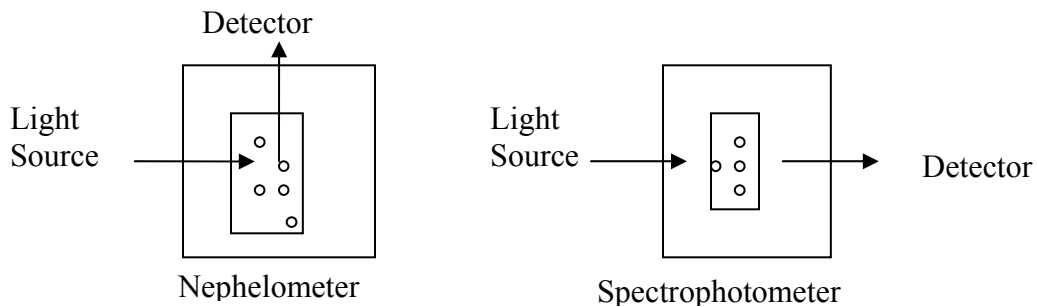
FSS = wt. after furnace - tare wt.  
 $= 1.3274 \text{ g} - 1.3255 \text{ g} = 0.0019 \text{ g}$  in 200 mL  
 $= 9.5 \text{ mg/L}$

VSS = SS - FS  
 $= 15.5 \text{ mg/L} - 9.5 \text{ mg/L}$   
 $= 6 \text{ mg/L}$

or you can use filter wt.

$= \text{wt of filter before furnace} - \text{wt of filter after furnace}$   
 $= 1.3286 - 1.3274 = .0012 \text{ g}$   
 $= 6 \text{ mg/L}$

One other important measurement that is discussed in the chapter is turbidity. Turbidity is also a measure of suspended solids. In this case the property measured is the scattering of light by solids suspended in water. Turbidity is often used as an easily monitored control parameter for monitoring solids in water. Turbidity should be measured by a Turbidimeter (or Nephelometer) which measures light scattered at 90° angle (see below). Alternatively (but not as accurately) you can measure the change in absorbance of light using a spectrophotometer as a surrogate. This method is subject to much interference because of absorption of light (depending on the light source) from dissolved particles in the water.



Turbidity is quantified by comparison of the light scattering (or absorption) of the sample to a standard curve developed using standard formazin suspensions. The units the results are expressed in are NTU = nephelometric turbidity units.

For this class it is also a good idea to review the section on nitrogen and phosphorous (P 60-63) and the information on gas transfer (p 64 – 70) may come in handy when we discuss the addition of oxygen to aerobic systems.

### **Organic matter**

In order to get a good grip on organic chemistry you would normally take at least two semesters of organic chemistry from the chemistry department. We don't have time to do this so I ask that you read the text and understand the material presented in the text. I would strongly recommend that if any of you want to go on in environmental engineering you should take some organic chemistry.

The minimal information needed to get by in this class is to understand how organic chemicals are measured.

### Measurement of Organic Compounds

1. Trace measurements - concentrations  $\cong 10^{-3}$  -  $10^{-12}$  g/m<sup>3</sup>
2. Gross measurements - concentrations  $> 1$  g/m<sup>3</sup>

### 1. Trace

Most trace analyses are instrumental; they use various instruments like the gas chromatograph, the mass spectrometer, high pressure liquid chromatography, and many others. Detection limits have improved greatly in the past few years and are now down to  $10^{-9}$  g/m<sup>3</sup> ( $10^{-12}$  g/L). There is not time to detail more about trace analysis of organic compounds in this course. Many of you will have the opportunity to learn more of this in other classes or as part of your research.

### 2. Gross

- a) chemical oxygen demand (COD)
- b) total organic carbon (TOC)
- c) total oxygen demand (TOD)
- d) biochemical oxygen demand (BOD)
- e) toxicity testing

Why so much interest in oxygen demand.

Historical - the addition of municipal waste effluents to streams, lakes, and rivers caused the death of the natural aquatic life in the water.

Why - the most efficient mechanisms for the metabolism of organic compounds is to couple this metabolism to the reduction of O<sub>2</sub>. Many of the bacteria are capable of performing this type of metabolism, and as long as oxygen is present, the bacteria will use it in their metabolism. The bacteria present naturally in the waste and receiving waters use up all of the oxygen in the water while degrading the organic compounds in the waste. This oxygen usage is dependent on the concentration of organic matter in the waste, and so in order to determine the effect a waste would have on the receiving water, engineers had to develop a gross measure of the "oxygen demand" a waste stream would have. This is an estimation of how much oxygen would be required to degrade the organic compounds in the waste. To do this you don't need to know the individual chemical makeup of the waste just the gross amount of oxygen required to degrade those chemicals.

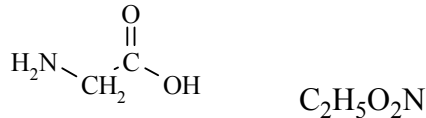
In this way the effects of the waste stream on the receiving water can be determined and you can treat the receiving water until the effect would no longer be detrimental. A demonstration of the oxygen demand concept can be found in the theory of the theoretical oxygen demand.

### Theoretical Oxygen Demand (ThOD)

If you know the chemical formula of the organic compound you can calculate the theoretical oxygen demand. i.e. the theoretical amount of O<sub>2</sub> required for complete oxidation of each element in the compound - the amount of oxygen present in the compound initially.

To do this you should write balanced equation for the oxidation reaction  
must balance atoms, oxidation states and charges

e.g. 200 g/m<sup>3</sup> of glycine



There are many ways people are taught how to balance equations. I usually balance C first then N, put in  $\text{H}^+$  to balance charge, then  $\text{H}_2\text{O}$  to balance H's and finally  $\text{O}_2$ 's to balance O.

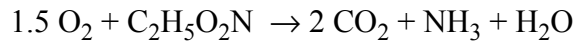


The balanced equation suggests that for every mole of glycine present you need 3.5 moles of  $\text{O}_2$  to allow complete oxidation. These results are usually converted to a weight basis and then to a grams of oxygen per volume of waste water.

$$\text{ThOD} = \left( \frac{3.5 \text{ mol O}_2}{\text{mol glycine}} \right) \left( \frac{32 \text{ g O}_2}{\text{mol O}_2} \right) \left( \frac{1 \text{ mol glycine}}{75 \text{ g glycine}} \right) \left( \frac{200 \text{ g glycine}}{\text{m}^3} \right) = 298.7 \text{ g O}_2/\text{m}^3$$

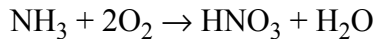
Theoretical carbonaceous oxygen demand

the amount of oxygen to convert organic to  $\text{CO}_2$  (leaving N as  $\text{NH}_3$ )



$$1.5 \text{ mol O}_2 \text{ per mol glycine} = 128 \text{ g O}_2/\text{m}^3$$

Theoretical nitrogenous oxygen demand

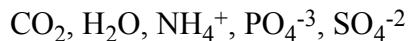


$$2 \text{ mol O}_2 \text{ per mol NH}_3 = 170.7 \text{ g O}_2/\text{m}^3$$

### Gross Organic Content Measurements

a) COD - organic compounds are oxidized with strong chemical oxidizing agent in acid. Silver sulfate is used as a catalyst to allow oxidation of even recalcitrant compounds. You start with known amount of  $\text{Cr}_2\text{O}_7^{2-}$  in excess of what is expected to be needed, measure what is left over at the end. Process converts all compounds to the oxidized state except  $\text{NH}_4^+$ .

e.g.



-takes about 3 hours

-does not differentiate between compounds that are non-degradable (i.e. will not effect oxygen demand of biological systems) and those that are degradable, so will overestimate the oxygen required when waste is degraded biologically.

- does not provide rate information so does not give any indication of how long it will take to get the waste degraded.

b) TOC - instrumental test

acidify sample to remove all  $\text{CO}_2$  present in the sample.

evaporate water off of sample  
oxidize catalytically to  $\text{CO}_2$   
measure  $\text{CO}_2$  produced with infrared analyzer

c) TOD - instrumental as well

some inorganic compounds are oxidized as well as organic ones  
platinum catalyzed oxidation chamber  
TOD is determined by the loss of  $\text{O}_2$  in the nitrogen-oxygen carrier gas

d) BOD - amount of oxygen used in metabolism of biodegradable organics

principle reactions in natural waters  
true test of oxygen demand on water  
takes time to get results  
CBOD - carbonaceous - meaning  $\text{NH}_3$  is a result  
NBOD - nitrogenous - meaning  $\text{NO}_3^-$ , or  $\text{HNO}_3$  is a result.

The BOD test was included as a lab in the prerequisite course CIVE 6391. I will not repeat the lesson here. Make sure that you read the text or review the 6391 lab material there will be BOD problems on your homework.

## Biological Contaminants

I will not cover this section of the chapter because we have covered most of it in the microbiology class. Table 2-23 (overhead) has some useful information about organisms resistant to environmental factors.

## Toxicity Measurements

Because there are so many toxic agents created by man that may be active at levels below the normal COD or BOD requirements for wastewater discharge, the EPA and local regulatory officials are requiring that toxicity tests be performed on industrial and some municipal wastewaters, as well as the usual BOD and/or COD tests and the tests for individual constituents.

The terminology used when considering toxicity testing is summarized in Table 2-29 (overhead). Table 2-30 lists some common test species used in the different toxicity tests. The choice of test species is frequently dependant upon environmental relevance. The species used should be relevant to the environment that the toxic species is expected to be present in. Examples 2-14 and 2-15 (overheads) go through data interpretation for you.

## Chapter 3 and 4.

This material will not be covered in any detail in this course. You should read and understand the materials discussing the variations in flow and constituents of wastewaters on your own. You should also understand the difference between reactor types (overhead from last text edition, Figure 4-2 in new edition).

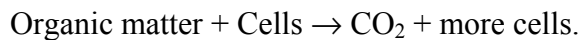
The section on modeling is covered in the environmental modeling course so will not be presented here.

## Chapter 7. Fundamentals of Biological Treatment

### Objectives of biological treatment:

1. Removal of soluble organic matter.

This is accomplished by exposing the main wastewater stream to biological action. The organisms utilize the soluble organic matter as a food source, thus removing it. The products of this are more cells and CO<sub>2</sub>. The reaction is often written as follows.



2. Stabilization of insoluble organic matter.

Insoluble organic matter can be removed from the main wastewater stream by entrapment in the biological matrix and conversion to stable products. The initial entrapment occurs in the main body of wastewater, while the further stabilization usually occurs in reactors designed to treat more concentrated streams of solids. The stabilization processes can be aerobic or anaerobic and involve holding the smaller solids stream for a longer period of time to allow more complete metabolism of the solids present.

3. Transform or remove nutrients.

4. remove specific trace constituents.

Biological processes are rarely used as stand alone processes. They are combined with various physical and chemical unit processes. Typical process flow diagrams are presented in Figure 7-1 (overhead).

### Biological Metabolism

The initial material in section 7-2 is more review from your microbiology class, read it on your own if you didn't take the course in sequence or if you just want the reminder. Table 7-1 and 7-6 (overheads) summarize some of the info succinctly.

Growth factor requirements

compounds (macronutrients) are needed in smaller amounts as major cellular constituents

Nitrogen is needed for proteins, some sugars and nucleic acids,

Phosphorous is needed for nucleic acids, and some metabolic cofactors

Sulfur is needed for proteins

K, Mg, Na, Ca, Fe, Cl, for membrane stabilization and some enzymes



Some compounds are needed in even smaller amounts (micronutrients) to allow the proper function of some enzymes (Zn, Mn, Mo, Se, Co, Cu, Ni, V, and W).

Most of these compounds are present in most natural and waste waters so we do not normally worry about having to add them except growing cultures in the lab. Some industrial waste waters may be unbalanced nutritionally (i.e. way more carbon than N or P (petrochemical), or way more N than C or P (meat processing). In these cases the nutrient imbalance must be addressed.

## **General Metabolic Mechanisms**

In order for organisms to metabolize compounds they must carry out chemical reactions assisted by enzymes produced by the cell. These reactions release energy from the compounds to specific energy carriers in the cell. The energy carriers transfer the energy to the cellular processes that require it.

Last semester we discussed.

- energy generation - central metabolic pathways, TCA cycle, electron transport
- pmf (proton motive force) - energy storage just like a battery
- ATP energy coupler (transfers energy to reactions that need it)
- NADH, NADPH carry electrons to and from reactions
- the capabilities of known types of enzymes
- Pathways for metabolism of specific compounds

We did not go into how we can convince organisms to grow on substrates that they are not used to or to grow under what would normally be toxic conditions. This material is not from your textbook.

## **Adaptation and Acclimation**

The adaptation or acclimation of microorganisms to toxic chemicals and new substrates is an important factor to consider when starting up new biological processes for the treatment of industrial wastes. A lot of the material from this lecture is taken from Martin Alexander's book "Biodegradation and Bioremediation" as well as other papers that I have collected on the subject.

The process of encouraging biological systems to adapt to new substrates for their sources of carbon and energy is called enrichment. The process that the organisms go through (learning to grow on new substrates) during the enrichment process is called adaptation or acclimation.

### ***Adaptation to Toxic Chemicals***

The adaptation of microorganisms to the presence of toxic components of a waste stream can occur in many ways. Biochemically the process usually involves changes in cell envelope structure or changes in enzyme systems so the cells will be able to function in the new environment. In some cases adaptation involves the removal of the toxic compound by some metabolic process. This is not true adaptation because the organisms don't grow until the

product has been removed. It is still a valid mechanism for organisms to survive under toxic conditions.

The process of causing microorganisms to adapt to the presence of toxins is usually accomplished by exposing the organisms to increasing amounts of the toxic agent.

One example of this is found in an attempt to develop a biological method to treat wastewater from a coal gasification plant. The wastewater contained both phenolic compounds and cyanide. A culture was developed to degrade phenolic compounds and then was exposed to different levels of cyanide. The first exposure shows that the organisms could only degrade phenol when up to 25 mg/L cyanide was present. When the cultures that survived the 25 mg/L exposure were allowed to grow for a while in the presence of 25 mg/L cyanide, then tested against higher concentrations they could survive up to 100 mg/L cyanide. Thus by allowing the culture to adapt to low levels of a toxicant, and increasing the levels of exposure slowly the culture was ultimately able to survive in conditions that were initially impossible.

There is usually some physiological limit to the amount of toxicant an organism can become acclimated to. In the case of phenol, this occurs when the phenol becomes so concentrated that it dissolves the cell membranes. The cyanide did not appear to have a physiological limit as the organisms were removing it from the medium.

### **Acclimation to New Substrates**

The first stage or acclimation is the stage in which the microorganisms learn to degrade the compound in question. This stage can last from hours to years, depending on the compound and the environment that is being examined. In most cases, if the environment has never seen the chemical the acclimation phase will be long. If the environment has been exposed to the chemical or one like it the acclimation phase may be shorter.

Several theories have been proposed to explain the acclimation of microorganisms to new compounds. These theories include:

Proliferation of small populations. This theory suggests that the microorganisms capable of degrading the new substrate are present in the natural population, but in very small numbers. The acclimation period, then, would reflect the induction of the appropriate enzymes and the increase in numbers of the organisms capable of growing on the new substrate. This would be reflected in low numbers and slow rates of degradation initially, then an increase in numbers of organisms and a corresponding rate increase.

Presence of toxins. This theory suggests that the presence of toxic chemicals suppresses biodegradation until some organism is capable of degrading or are not inhibited by the toxin develop.

Predation by protozoa. It has been observed that when protozoa (which normally feed on bacteria in waste water systems) are inhibited the lag times seen for acclimation to new materials are decreased dramatically. It is presumed that the grazing of the protozoa on the bacteria is keeping the population of the acclimating bacteria so low that noticeable degradation does not occur.

Appearance of new genotypes. Natural mutations occur about once every million cell divisions in a bacterial culture. If the mutation results in a new genotype with altered enzymes that are capable of degrading the new substrate it will give the culture a selective advantage. Studies concerning the evolution of enzymes suggest that small changes in the substrate binding site of the enzyme will allow an enzyme to bind a new substrate and then perform its chemical reaction. Thus, whole new enzymes are not made from scratch, but old enzymes end up being modified to new substrates.

Diauxie In many cases some substrates are degraded preferentially over other substrates. Catabolite repression is a good example of this. If glucose is present in a media, many organisms will degrade only it until it is gone then they will begin to degrade the other substrates. In some cases the organisms will degrade the first substrate and then after a lag period start to degrade the second substrate.

Enzyme induction and lag phase This theory presumes that the acclimation period is due to enzyme induction in a population that is already present. The enzymes are only formed when the substrate is present. When you put organisms capable of degrading a substrate, but have been degrading something else, back into the substrate, it usually takes them a while to recognize the new substrate and turn on the appropriate enzyme systems.

In most cases several of the theories will apply to the overall explanation of acclimation. The use of plate counts on selective and non-selective media, and characterization of colonial morphology will help to show whether growth of a small population or appearance of new microorganisms is involved in the acclimation process.

### **Growth**

The second phase, or as I call it growth or acceleration phase, is the phase in which the organisms that have acclimated to the degradation of the compound are allowed to increase in number and possibly in specificity. In this stage the numbers of organisms capable of degradation of the chemical increase and thus the rate of the degradation. In some cases the constant exposure to the chemical causes an increase in the production of the enzymes responsible for the metabolism of the compound so the rate can increase even more than allowed for by the increase in number.

### **Techniques to Study Acclimation and Growth on New Substrates**

There are two basic enrichment culture techniques. One technique involves the exposure of the inoculum to be used to only the chemical of interest. In this case, only the organisms capable of degrading the compound will grow. For toxic substrates, this may lead to long acclimation periods, as the organisms resistant to the chemical and capable of degrading it may be present only in low numbers initially if at all. This technique is often successful though when low concentrations of the chemicals are used (mg/L range). The resultant cultures will be able to degrade the chemical with no further additions other than the basic nutrients.

The second enrichment technique uses the addition of an easily degradable substrate along with the compound of interest in order to stimulate a lot of growth. This should allow organisms in low numbers to increase to numbers that will be capable of utilizing the chemical of interest more easily. This method is also successful in some cases.

The major drawbacks from the second method are:

- a) the more easily utilized substrate will stimulate growth in general, not necessarily only of the organisms capable of degrading the chemical, this may lead to competition for available nutrients,
- b) the resultant culture may become dependent on the addition of the other carbon source for good activity,
- c) the culture may exhibit Diauxie, that is only use the chemical of interest if the more easily utilized substrate is used up, thus increasing the acclimation period.

### **Activated Carbon Addition**

In most cases for industrial waste treatment, exposing the organisms to the waste for long periods of time will eventually lead to enrichment on the new substrate. In some cases this could take a long time, and the substrate will not be removed from the water until this time. One engineering practice that can help in this process is the addition of powdered activated carbon to the wastewater. The activated carbon binds the organic chemicals of interest and removes them from the water. The carbon settles with the biological sludge and exposes the organisms in the sludge to a higher concentration of the compound (usually beneficial) and for longer periods of time. The benefit is two-fold.

1. The compound is removed from the wastewater so the treated water is in compliance,
2. The organisms can acclimate to the new substrates at their own rate

Eventually the activated carbon can be weaned out of the system, but a large amount of sludge recycle is required initially.

## **Important Microorganisms**

**Readings** Text Chapter 2-8.

During the activated sludge process several microbial species, including both eukaryotes and prokaryotes, have been found. Since there are so many organisms present, I don't want to go through all of them. I would, however like to mention some of the important organisms.

### ***Bacteria***

You have learned a lot about bacteria in the Micro class but I would like to take this time to present some information concerning some specific organisms that are beneficial or troublesome to waste water treatment.

## **Pathogenic bacteria**

Table 2-24 (overhead) summarizes the pathogens currently of concern today. We do not usually test waters for all of these organisms but we use indicator organisms to determine if it is possible that animal or human waste has contaminated the water (thus the presence of pathogens is likely).

Normal flora of the human intestinal tract (*Escherichia coli*) and pathogenic bacteria from the human intestinal tract (*Salmonella*, *Shigella*) are common constituents of municipal wastewaters. These bacteria do not survive well and are usually removed from the water either by attrition or due to scavenging by protozoa or rotifers. The presence of these organisms in receiving waters is indicative of sewage contamination. To be safe many treated waters are chlorinated as a disinfection procedure.

## **Bacteria with degradative capabilities**

### **Aerobes**

There are many aerobic bacteria present in an aerobic wastewater treatment system. Many of these will be able to carry out some of the metabolic processes needed to treat the waste. None will be able to carry out all of the metabolic processes necessary. Thus, a mixed culture of many organisms is required to treat a complex waste stream. One large group of organisms called Pseudomonads includes the majority of the organisms that carry degradative genes. The Pseudomonads are classed as Gram negative straight or slightly curved rods with polar flagella. True Pseudomonads never show any fermentative capabilities, so are limited to aerobic or nitrate reducing conditions. Some example genera of organisms that are included in the Pseudomonad group are *Pseudomonas*, *Comamonas*, *Burkholderia*, *Zooglia* and *Gluconobacter*. For the most part the degradative genes are carried on plasmids, and can be transferred to individuals within a species and in a lot of cases within species and maybe genera within the Pseudomonad group. Other important genera that carry degradative genes are *Aeromonas* (just like *Pseudomonas*, but will ferment), *Flavobacteria* (also very similar to *Pseudomonas*), and *Bacillus* (Gram variable endospore forming rod, common soil organism, variety of metabolic capabilities).

### **Zooglia**

*Zooglia* is a bacterial genera that grow and form a lot of extracellular material with adherence characteristics. *Zooglia* are classed in the Pseudomonad family. These organisms form the basis of the floc, which is the substance that the other organisms attach to. Without the floc, the cells would not settle and thus would wash out of the reactor. The floc is sensitive to solids retention time in the reactor. If you don't have enough recycle or if you flow too fast, the floc does not form and the cells all wash out.

The floc also serves the purpose of adsorption of the organic compounds from the water. The hydraulic detention time in most municipal reactors is usually 5-10 hours, this is too short a time for the complete biological degradation of the organic compounds present. The main mechanism of contaminant removal may be the initial adsorption of the compounds into the floc. A lot of the degradation of the compounds occurs in the floc during and after the activated sludge system.

Aerobic or anaerobic digestion of the solids in the sludge often serve as the real systems in which a lot of the organic compounds are degraded.

### **Anaerobes**

In anaerobic sludge digesters again we see a community of microorganisms. This community is much more interactive than that in activated sludge. Under anaerobic conditions, the large compounds must be first fermented to smaller compounds, which are then utilized, by acetogens and methanogens ultimately forming CO<sub>2</sub> and CH<sub>4</sub>. The methanogens and acetogens must remove the fermentation products so they do not accumulate and back up the metabolism of the larger molecules. (More on this later). Of the anaerobes, the most important organisms that carry out degradations or alterations of the large compounds are the *Clostridia*. These are anaerobic endospore-forming rods that carry out many industrially important fermentations and are very predominant in anaerobic waste water treatment.

### **Filamentous Microorganisms**

Organisms that grow as filaments are an important part of the floc and can also interfere with the proper formation of the floc and cause bulking when present in too large a number. This is detrimental to proper sewage treatment as it interferes with secondary clarification and thickening. The names of some of these organisms are *Micothrix*, *Sphaerotilus*, *Beggiatoa*, *Thiothrix*, *Nocardia*, *Lecicothrix*, *Geotrichum*. These organisms are always present in sludge in small numbers, but when the reactor becomes nutrient stressed, the filamentous organisms have a growth advantage under these conditions, so will proliferate. (Low O<sub>2</sub>, P, C, N, can all be causes). We will talk more about this when we talk about activated sludge.

### **Fungi**

Fungi are single or multicellular eukaryotic organisms that do not produce chlorophyll. Fungi are aerobic heterotrophs and are highly resistant to adverse environments e.g. Fridge mold, jam mold etc. They are important in composting operations and in treatment of some industrial wastes. The most important fungi for waste treatment is *Phanerochaete chrysosporium*. This is the white rot fungi that produces peroxidase that is so effective in degrading lignin.

### **Protozoa**

Single celled eukaryotic organisms. Some protozoa are parasitic organisms that are present in municipal sewage so are present in the treatment tanks. Such organisms are disease causing agents, so part of the treatment of sewage is aimed to kill these. e.g. *Cryptosporidia*, *Giardia*. Some protozoa scavenge and digest bacteria, these are important for controlling bacterial pathogens as well as the overgrowth of other bacteria in a treatment system. e.g. *Paramecium*, *Vorticella*.

### **Rotifers**

These organisms are important to wastewater treatment because their main source of food is bacteria. It is believed that rotifers scavenge the free floating bacteria, thus reducing BOD and also the numbers of pathogenic organisms in the water.

## **Viruses**

Viruses are disease causing agents that are present in sewage and are removed during sewage treatment by adsorption to the floc.

In industrial treatment processes the characteristics of the waste will select the population of organisms present in the waste. A diverse waste will select diverse types of organisms, while a defined water stream may narrow the type of organisms present due to the selection of organisms with specific degradative capabilities. If *Zooglia* are effected by the waste stream characteristics then the process will have to be altered because a floc is necessary in the activated sludge process. This is part of the reason treatment methods with solid phase support for prevention of bacterial washout are very popular with industrial wastes. Some kind of floc or support is necessary to prevent washout of the active cells. A rich carbon source is a benefit in that it allows the formation of extracellular polymers which provide a glue like substance to allow organisms to stick together (floc) or to a solid support.

## **Microbial Kinetics and Materials Balances**

In order to design and operate a waste water treatment plant you must be able to calculate things like flow rates, residence time and reactor sizes. These things are often based on the mathematical analyses of what the capabilities of the biological populations are. Most kinetic studies are performed on batch reactors, then the results are extrapolated to continuous flow systems. I will present a brief review of the subject, since Dr. Chellam gives excellent treatment to the subject in the environmental modeling course, but I have a few points specific to Microbial kinetics that I feel strongly about.

### ***Reaction Kinetics***

#### **Rate and order of reactions**

The rate and order of the reaction is the term used to describe the disappearance or formation of a chemical species.

Units for rate measurements  
homogenous - mol/L/t or g/L/t  
heterogeneous - mol/m<sup>2</sup>/t

Reactants have negative rates of reaction (concentration decreasing with time) and products have positive rates of reaction (concentration increasing with time).

In biological systems (and most others) at a constant temperature, the rate of reaction is typically a function of the concentration(s) of the reactants, the biomass (or numbers of cells and enzymes). The rates are also typically saturable. That is the rate can increase to a certain point

as you increase the concentration but you will reach a point where the system is working as fast as it can and can't get any faster.

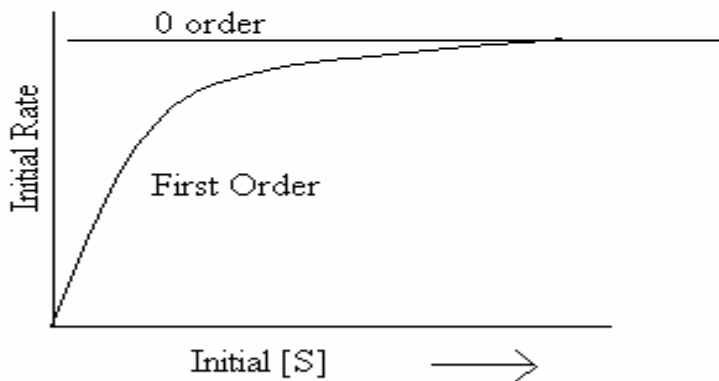
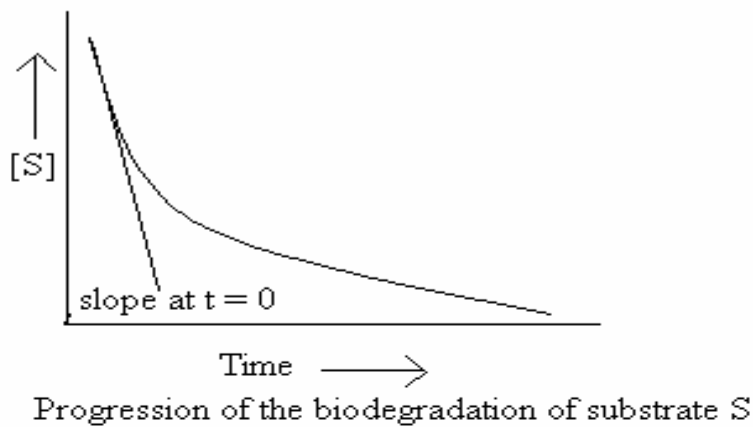
0 order - means independent of concentration

1st order - means dependent on concentration of 1 species (when concentration goes up so does rate)

2nd order - dependent on concentration of two species

saturation - discussed more later

autocatalytic - rate is function of product concentration



Effect of initial substrate concentration on initial rate

To further explain saturation we need to discuss saturation of enzymes. As you know, all biological reactions are carried out by enzymes. Each cell only has a finite number of enzymes



present. These enzymes can only turn over the substrate at a specific rate ( $r$  or  $v$ ). Once all of the enzymes are busy an increase in substrate cannot increase the reaction rate because the enzymes are working to their maximum ( $V_{\max}$  or  $V$ ). Thus the reaction rate appears independent of the concentration. Below this saturation level of concentration the enzymes are not working at maximum speed, so increases or decreases in concentration do effect the rate of the reaction. The limitation here is the diffusion of the chemical into the enzyme active site. In many cases a system is both saturated in enzyme function and cell number (i.e. you can't improve the rate by adding more cells either). Such a system is said to be at its carrying capacity.

Each enzyme will have its characteristic  $V_{\max}$  and saturation concentration at which further increase in concentration does not effect the observed reaction rate. The number of enzymes present will also change the saturation concentration and the  $V_{\max}$ . More enzymes will require higher amounts of substrate to saturate and will use the substrate at a rate that is apparently faster. That is why we always report the kinetic coefficients as a measure of enzyme concentration for pure enzyme studies or using some kind of biomass measurement to qualify kinetics in a culture system.

The model usually used to describe enzyme kinetics is the Michaelis-Menten rate law. This rate law is in the form of an equation that describes the rate of substrate degradation in terms of the concentration of substrate and the concentration at which 1/2 the maximum rate is achieved.

#### Michaelis-Menton rate law

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

where  $v$  = velocity or rate at a specific concentration (mol/L/t)

$[S]$  = substrate concentration (mol/L)

$V$  = maximum rate of reaction (mol/L/t)

$K_m$  = substrate concentration which gives half maximal reaction velocity (mol/L)

when  $S \gg K_m$  then  $v=V$

when  $S \ll K_m$  then  $v = \frac{V}{K_m}[S]$

The bacterial or enzyme concentration must be kept constant as  $V$  is a function of enzyme or bacterial concentration. The constants determined must also be qualified with the amount of cells or enzymes present as described above.

#### **Growth Kinetics**

The growth of microorganisms can be described by the same type of equation. Microbial growth rates are dependant on the concentration of a limiting substrate. There will always be one substrate that is limiting, and this will control the rate of microbial growth. For microbial

growth, the term  $v$  is replaced by  $\mu$  and  $V$  is replaced by  $\mu_m$ . The equation describing microbial growth is the Monod equation.

Monod Equation

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

where

$\mu$  = specific growth rate (time<sup>-1</sup>)

$\mu_m$  = maximum specific growth rates (time<sup>-1</sup>)

$S$  = concentration of growth limiting substrate in solution (mass/unit volume)

$K_s$  = half-velocity constant, concentration of the limiting substrate at 1/2 the maximum growth rate (mass/unit volume)

If we want to calculate substrate degradation rates and incorporate microbial growth we can use the modified equation.

$$r_{su} = -\frac{\mu_m X S}{Y(K_s + S)}$$

where  $r_{su}$  = substrate utilization rate (mol/L/t)(mg/L/t)

$\mu_m$  = maximum growth rate (mg cells/d)

$X$  = concentration of microorganisms (mg/L)

$S$  = concentration of growth limiting substrate (mol/L) (mg/L)

$Y$  = yield of cells (mg cells/mg substrate)

$K_s$  = half saturation constant (mol/L) (mg/L)

### **Biological degradation rate studies.**

One of the most common mistakes found in the study of biological reaction rates is due to a poor setup of experimental conditions. Setting up a reaction at a specific concentration and following the disappearance of the substrate with time, and then integrating the changes in the rate over the time and correlating it to the concentration at that time does not give acceptable results. During bacterial degradation rate studies you usually also include the amount of cell mass or protein concentration (indicative of enzyme concentration). Since the rate depends not only on the concentration of the compound but on the amount of enzyme present.

A proper biological degradation study is performed by inoculating several different concentrations of substrate with equal amounts of cell mass (or protein) and taking the initial rate. Taking these rates and plotting them against the concentration they were observed in gives you a plot like the one in the previous figure.

As the figure shows. The data approach an asymptote. The determination of  $V$  (asymptote) and thus  $K_m$  ( $1/2$  asymptote) can be done by approximation, using this plot, or by manipulating the equations to obtain linearized plots. There are several ways to linearize this data.

### Linearized forms of kinetics equations

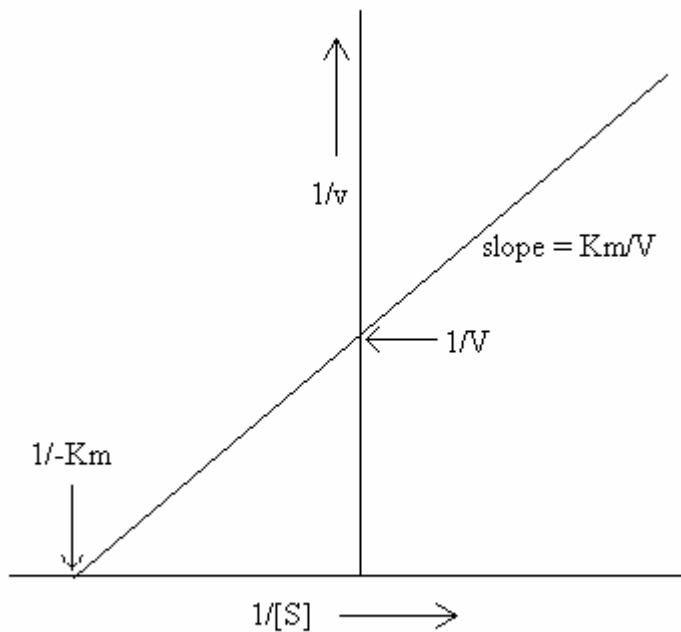
1. Lineweaver-Burke equation

$$\frac{1}{v} = \frac{1}{S} \frac{K_m}{V} + \frac{1}{V}$$

slope =  $K_m/V$

y-intercept =  $1/V$

x- intercept =  $1/-K_m$



Lineweaver - Burke Plot of Kinetic Data

2. Eadie-Hofstee

$$\frac{v}{S} = -\frac{1}{K_m} v + \frac{V}{K_m}$$

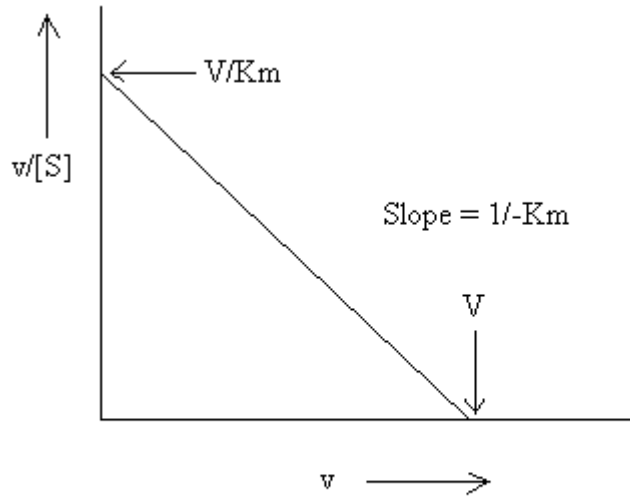
Plot  $v/S$  vs.  $v$

slope =  $-1/K_m$

y-intercept =  $V/K_m$

x-intercept =  $V$

This plot is a little more resistant to errors, since only one of the plots is a reciprocal plot. although the term  $v$  is in both axis, which is not recommended.

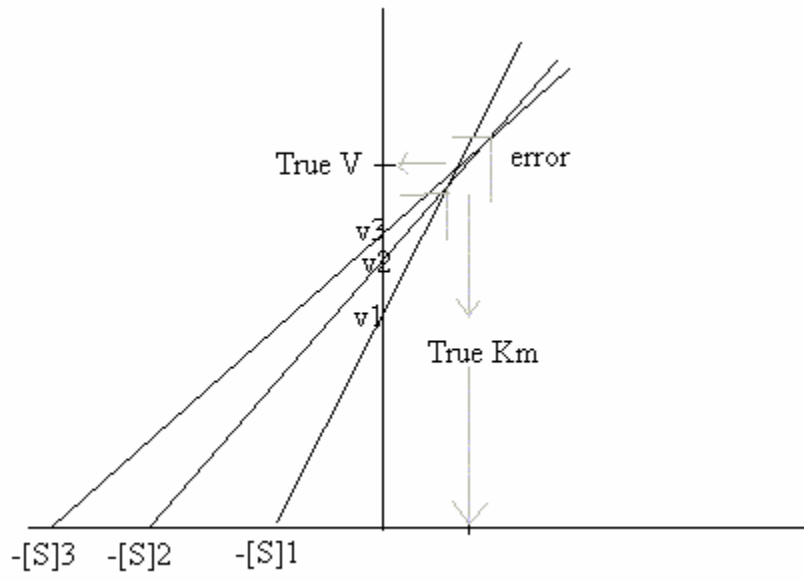


Eadie-Hofstee plot of kinetic Data

### 3. Direct Linear Plot

This plot is obtained by plotting the data from several experiments directly. The  $v$  data are points on the  $y$  axis. The  $S$  data are plotted as negative points along the  $x$ -axis. When lines are drawn from the points on the  $x$ -axis through the points on the  $y$ -axis the point where they intersect describes  $V$  and  $K_m$ . The  $v$  is the extrapolation back to the  $y$  axis of the intersection point, and the  $K_m$  is the extrapolation down to the  $x$ -axis from the intersection point. (overhead)

This plot can also be used for error determination. The true  $K_m$  and  $V$  are taken from the median point where the experimental data cross. The extremes in each direction give the error.



Direct Linear Plot of Kinetic Data.