C & EE 255B. Professor Michael K. Stenstrom

In this course we will learn principles and empirical results that will enable us to understand and eventually design treatment processes that in some way use microorganisms to improve water quality. Generally, we will be concerned about wastewaters and their treatment, although many of the principles will apply to water treatment or other processes that use microorganisms. For example, growth kinetics and stoichiometry could be applied to pharmaceutical manufacturing or beer production.

This is the first year we are using a new text book, *Environmental Biotechnology: Principles and Applications.* In previous years we have used *Wastewater Engineering,* which is the most popular textbook ever written for environmental engineers. *Wastewater Engineering* is in its 3rd edition and a 4th edition is nearing completion. The approach in this very popular book is to combine many design principles with appropriate theory to produce a complete treatment of all aspects of wastewater treatment. As a result, there are many items that are not needed in a single course. Often I spent time telling students what not to study, as opposed to what we will cover. *Wastewater Engineering* is still the best single reference for a practicing environmental engineering. Do not sell your old copy!

Our new textbook takes a different viewpoint. Much more theory is presented with correspondingly less emphasis on practice. Moreover, the authors are much better versed in theoretical issues than practical issues. Often they will suggest the reader refer to other literature "to gain a more in-depth understanding." Since more emphasis is placed on theory, many more principles from microbiology are included. The text includes several chapters on microbiology, and the chapters are written for engineering students who have more specific needs that an undergraduate student taking a general micro course. You will find these chapters are a very useful summary of useful information. They provide definitions that would be found in a lower division book in paragraphs next to information that would be found only in advanced texts. The authors' work will save you lots of time and give you a better understanding of the material.

I have assigned chapters for various lectures. Generally, there will be much more material in the reading assignments that are contained in the lectures. You should use the lectures as a guide to what is important (at least for this course). I will also provide several sets of notes and these will generally be placed on my web site (www.seas.ucla.edu/stenstro) in PDF format.

Microbiology Chapter 1.

The authors state that microbiology is very complicated, so complicated in fact that it is too difficult to quantify. Engineers generally think that if you cannot quantify something, that is to say, describe it with differential equations and other mathematical tools, it is less valuable, "soft," qualitative, and generally less valuable. The authors say differently: microbiology is more valuable because it is difficult to quantify. Therefore observations are more valuable.

As engineers we enjoy topics where theory provides a logical, mathematical sequence for us to learn. We do not need to memorize because we can figure it out from theory. Microbiology has similar theories, but we will need to memorize many definitions, reactions and concepts before we can benefit from the theory.

Cells. Fundamental building blocks of life forms. They are capable of growth and reproduction. They are highly organized, and composed mostly of elements C, H, O, N, P, and S, and exist in chemically reduced states (carbon is bonded more frequently with H (+1 oxidation state or valence) than O (-2 oxidation state), so the oxidation state of C is usually closer to –4 than +4). Carbon dioxide (CO_2) is the most oxidized form of carbon (+4 oxidation state), and methane (CH_4) is the most reduced form of carbon (-4 oxidation state).

Compounds undergoing oxidation (increasing oxidation number) generally release energy or heat, while compounds undergoing reduction require energy. Therefore growing cells must manufacture reduced compounds and will require an energy source. We can use oxidation/reduction chemistry to help us understand both stoichiometry and kinetics of microbial reactions.

Cell parts

Cells have many parts but these are the most important ones.

C*ell membrane.* All cells have a membrane and the membrane acts like a selective conduit for compounds that need to be transported into the cell (substrates, nutrients, electron acceptors) or out of the cell (waste products).

Cell wall. Not all cells have a wall. The wall provides rigidity to the cell and acts like a structure as opposed to a membrane.

Cytoplasm. The contents of the cell, which is mostly water but also includes the molecules that allow it to function.

Chromosomes are special, complex molecules that contain the genetic code, a sort of "blueprint," for the cell and its functions.

Ribosomes are complex molecules that convert genetic code in catalysts to promote the chemical reactions required to carry out the cell's functions.

Enzymes are catalysts that carry out the needed chemical reactions.

Domains

There are three domains that comprise all organisms. Bacteria have two domains – prokaryotes and Archaea. They live only as single celled organisms with chromosomes floating around in the cytoplasm. They are generally simpler organisms, but that does not mean they are less important. Eukarya maybe single or multicellular and contain their chromosomes in a nucleus. The scientific community has recently recognized Archaea as a separate domain from bacteria.

Figure 1.1 on page 4 of the text shows a "tree" based upon RNA sequencing of the three domains (left branch depicts bacteria, middle branch Archaea and the right branch are Eukarya. Note that we are Eukarya as well as simple organisms such as a paramecium.

Most of our work will focus on bacteria (biological treatment, such as the activated sludge process), Archaea (anaerobic digestion and other methane producing processes, and a few Eukarya, such as algae and *protozoans* (single, eukaryotic cell organisms, that grown on organic carbon).

Taxonomy & Phylogeny

We need to describe organisms in order to share information with other investigators and engineers. We have to adequately describe the microbial process in order to reliably reproduce it at other locations or in other designs.

Taxonomy is the classical method and relies on observable properties, such as shape, size (i.e., its appearance or *morphology*), or its reaction to certain chemical or environmental conditions (ability to absorb a chemical stain or ability to grown on a specific substrate). Collectively these properties are called a *phenotype*.

Phylogeny is a newer method of classification using molecular biology to analyze a cell's DNA or RNA. This technique will ultimately be more reliable and easier that taxonomy. The most frequently used approach is to analyze a cell's 16S rRNA. These methods are still developing and will ultimately be much more powerful. They are a little like a fingerprint. The fingerprint will help us identify a cell as belonging to a particular species, but they do not tell us what the species is capable of doing. For that we will rely on taxonomy. As a way of explanation, a fingerprint can identify a person, but will not tell us, by itself, if the person is a criminal or not.

The smallest unit for classification using taxonomy is *species.* A species is a group of organisms or collection of strains that have sufficiently similar properties to warrant

being grouped together. *Strains* are subgroups that have some property that is measurably different from other members of the species, but not so different that they should be considered different species. Groups of similar species are called *genera* or *genus* (singular). Collections of genera with similar properties are called *families.*

Naming. Organisms are generally named with the genus and species name. The genus name is first, followed by the species name. The genus name is first and is always capitalized and the species name is second and is never capitalized. The entire name is written in *italics* or underlined. The genus name can be abbreviated. For example, *Escherichia coli* or *E. coli*, and often the species is called "coliforms" in a nonscientific fashion.

In the past, species were discovered through a difficult process called isolation. The organism was grown in a fashion to select individuals that would represent a single species. The result was usually called a pure culture, meaning that only one species and/or one strain of organism was present. Over the years there have been many discovery of species only to learn later than the isolation was faulty. As a result, some species have disappeared! Taxonomy is difficult and inexact. The newer phylogeny methods will help avoid such problems in the future and will undoubtly change many current species classifications.

Morphology of bacteria includes the size and shape.

Shapes

Coccus – spherical
Diplococci – pairs of spheres
Streptococci – chains of spheres
Staphylococci – large groups of spheres
Sarcina – packets of eight spheres
Bacillus – cylindrical
Spirillum – spiral shaped.

Size

Cocci range in diameter from 0.5 to 5 microns (μmeter). Bacillus are generally 0.5 to 2 microns in diameter by 1 to 5 microns long. Filters for disinfection (removal or inactivation of disease causing organisms) must generally reject particles larger than 0.5 microns.

Morphology is important because we desire bacteria that grow in compact units or clusters and form even larger clusters called flocs. Bacteria that grown in strings or filaments are particularly troublesome.

Chemical Composition

Tables 1.2 shows the composition of prokaryotic cells. Many different investigators have measured various compositions. For environmental engineering work, we make special classifications for the cultures we grown in treatment plants. The first is based upon the type of suspended solids (SS) – total (TSS), volatile (VSS) and non-volatile (NVSS). Suspended solids or total suspended solids (volatile plus non-volatile) means the dry residue that remains on glass fiber filter paper (generally openings of 0.7 microns) after drying at 103^{o}C. If the residue is burned or "flashed" or "ashed" at 550^{o}C in a muffle furnace, the remaining material or ash is the non-volatile suspended solids . The volatile suspended solids are composed of organic carbon compounds and some nutrients such as nitrogen. The NVSS includes minerals and salts such as calcium carbonate and silica.

The chemical composition of the volatile suspended solids (VSS) is usually close to $C_5H_7NO_2$. We almost always use this empirical chemical formula for cells in our work. Note that it is an empirical formula, meaning that it is experimentally observed, and does not represent the actual formula of the cells. It would be impossible to determine a true formula for cells, since there are millions of compounds composing cellular material.

We use VSS for quantifying microorganism mass for several important reasons. Firstly other methods are generally too complicated and expensive, and provide little useful additional information. Secondly, the amount of minerals (sand, etc) and carbonates formed during treatment is much greater than the true mineral content of the cells. So called "activated sludge" may be composed of 10 to 40% NVSS, which participate in no reactions. Therefore VSS is usually the best indicator of microorganism mass.

Microbial Growth

We are most interested in quantifying microbial growth. It would be most useful if we could count viable cells, but this would be impracticable. We use VSS and we recognize that the actual number of cells may be incorrectly represented (cells of a single species will not have the same unit mass during all phases of growth and environmental conditions).

Bacteria generally divide by binary fission. Some cells grow by producing multiple spores that produce new organisms. *Nocardia*, an important species for environmental engineers, grow in long threads called filaments. The filaments can break and each segment can produce a viable offspring. There are also budding bacteria, which produce a stalk that eventually gives rise to a new cell.

There is also a type of reproduction that requires exchange of genetic material between bacteria (sexual reproduction). In this case, genetic material on a *plasmid* (a plasmid is a DNA containing molecule, smaller than a chromosome) is exchanged between cells. In this fashion, bacteria can acquire new abilities, such as resistance to an antibiotic or ability to degrade a different substrate.

Energy and Carbon for Growth

We characterize bacteria based upon the way they obtain energy and carbon for growth. The simplest classification is photosynthetic (obtaining energy from chemical reactions driven by light) or chemotrophic (chemical reactions). Algae (eukarotic) and cyanobacteria (formerly called blue-green algae) are photosynthetic.

Bacteria that obtain their carbon for growth from organic compounds are heterotrophic. Those that obtain their carbon from CO_2 are autotrophic. We can further refine the descriptions as follows:

Photoautotrophic –cyanobacteria, algae and other plants, obtaining their energy from sunlight and their carbon from CO_2.

Chemoautotrophic – obtaining their carbon from CO_2 but their energy from chemical reactions, such as nitrifiers (they convert ammonia to nitrite to nitrate).

The source of energy and type of carbon often relates to the terminal electron acceptor used by the bacterial. Aerobic bacteria use oxygen as their terminal electron acceptor. Anaerobic bacteria and Archaea use some other compound, such as an organic acid or CO_2. Environmental engineers use a special classification called *anoxic*, which means using nitrate (NO_3^-), nitrite (NO_2^-), or sulfate ($SO_4^{=}$) as a terminal electron acceptor. *Facultative* bacteria can use oxygen or another electron acceptor, depending upon environmental conditions. Other bacterial may be *obligatory* aerobic (strictly using oxygen as an electron acceptor), or obligatory anaerobic (oxygen is generally toxic).

Temperature ranges

Microbiologists have divided environmental temperatures into four ranges:

Psychrophilic	-5 to 20^{o}C
Mesophilic	8 to 45^{o}C
Thermophilic	40 to 70^{o}C
Hyperthermophilic	65 to 110^{o}C

Most of our processes work in the mesophilic range. A notable exception is thermophilic anaerobic digestion, which operates at the lower end of the thermophilic range.

1. Definitions

ADP
aerobes
ammonification
anaerobes
anaerobic
anoxic
archebacteria
ATP
autotrophic
bacillus
bacteria
catabolite repression
cell membrane
cell wall
cells - eucaryotic
cells - procaryotic
chemoautotrophic
chemotrophic
coccus
commensalism
denitrification
diplo
enzyme induction
eubacteria
facultative anaerobes
feedback inhibition
fermentation
gram negative
gram positive
gram staining
halophiles
heterotrophic
inhibition, Feedback
interaction among organisms
mesophillic
methanogens
methanogensis
microbial interaction - neutral
microbial interaction -positive
microbial interaction - antagonistic
mutualism
NAD
NADH
nitrification
nitrogen fixation
obligatory
oxic
oxidation
pathogenic
photosynthetic
phototrophic
protozoa
psychrophilic
reducing power
reduction
respiration
spirillum
staphylo
strepto
synthesis
thermophiles
thermophillic
virus

2. Cycles. Be able to diagram the carbon, oxygen, nitrogen and phosphorus cycles.

C_6H_6O