Wastewater Microbiology & Process Control

Toni Glymph, Senior Environmental Microbiologist
Metropolitan Water Reclamation District of Greater Chicago
Auralene.Glyphm@mwrd.org
Microorganisms mixed with wastewater (mixed liquor) in the presence of oxygen, consume biodegradable materials.

Wastewater from homes, industry, and sometimes storm water enter the WRP via interceptors.

Primary tank floats oil & grease and removes settleable solids.

Bar screens trap large debris.

Water is slowed down to allow grit to settle out.

Primary solids are pumped to solids handling/processing.

Secondary solids are wasted to solids handling/processing.

Final treated water is discharged to the waterbody.
Wastewater Microbiology

- The wastewater treatment process is a biological process.
- In order to properly evaluate this process we should incorporate biological tools.
- One of those biological tools is the microscope.
Tool: *The Microscope*

- **The Objectives:**
  - 10 X objective (magnifies 100 times)
  - 20 X objective (magnifies 200 times)
  - 40 X objective (magnifies 400 times)
  - 100 X objective (magnifies 1000 times)
    - oil immersion lens
  - This objective must be used with immersion oil
Introduction to Microscopy

- Brightfield Illumination
  - Light rays pass through the condenser which directs the rays through the specimen.
  - Not good for observing live samples. There is little contrast between the water and the microorganisms.
Introduction to Microscopy

BRIGHTFIELD ILLUMINATION
Introduction to Microscopy

- Phase Contrast Illumination
  - Uses a special condenser that allows certain structures to stand out from other less dense parts of the cell and from the surrounding fluid.
  - Cell shape and structure are more visible.
  - Phase contrast is more suitable for observing live microorganisms.
Introduction to Microscopy

PHASE CONTRAST ILLUMINATION
Introduction to Microscopy

Brightfield

Phase contrast
Introduction to Microscopy

- Darkfield Illumination
  - The light does not pass through the specimen but hits only the sides of the microorganisms.
  - Displays the background as black and the organisms as white
Introduction to Microscopy

DARKFIELD ILLUMINATION
Introduction to Microscopy

- **Differential Interference Contrast (DIC)**
  - Specially designed prisms recombine split beams of light
  - Gives a 3-dimensional appearance
Introduction to Microscopy

DIFFERENTIAL INTERFERENCE CONTRAST ILLUMINATION
Introduction to Microscopy

DIFFERENTIAL INTERFERENCE CONTRAST ILLUMINATION
Introduction to Microscopy

Brightfield

Darkfield

Phase Contrast

DIC
Introduction to Microscopy

Electron Microscopy
- Uses beams of electrons instead of light.
- Used to examine smaller objects such as viruses or internal structures of cells.
Introduction to Microscopy

ELECTRON MICROSCOPY
Introduction to Microscopy

*Slide Preparation*

- **Wet Mount**
  - Allows living organisms to be observed as they appear in the wastewater environment.
  - Cell measurements can be taken and cell shape is more accurately determined.
Introduction to Microscopy

*Slide Preparation*
Introduction to Microscopy

*Slide Preparation*
Introduction to Microscopy

Sample Collection

- The sample should be collected from the discharge end of the aeration basin.
- Slides should be made from a fresh sample of mixed liquor.
- If the sample is collected and cannot be examined right away, the sample should be aerated.
Microbiology of Activated Sludge

- Activated sludge is a mixture of microorganisms that come in contact with and digest bio-degradable materials (food) in wastewater.
- Some type of microorganism will always grow in the system. The organisms that are best suited to the environment will dominate.
Microbiology of Activated Sludge

- Bacteria (95%)
- Protozoa (4%)
- Metazoa (1%)
Bacteria

- Bacteria are amazing complex and are among the most abundant organisms on Earth.
- They can be found on our skin, in our mouths, in our intestines, in our food and drinks, in the air, in water, and in soil.
- Bacteria are introduced into the treatment system via surface runoff, wash water and feces.
Bacteria

- Bacteria are small, simple, one-celled microorganisms.
- They have one of three basic shapes
  - Coccus (spherical)
  - Bacillus (rod-shaped)
  - Spirillum (spiral or corkscrew shaped)
Bacteria
Bacteria

- Bacteria are classified in many ways
  - **Aerobic** – *require oxygen for growth and maintenance*
  - **Anaerobic** – *cannot tolerate oxygen*
  - **Facultative** – *prefer oxygen but can live without it*

- The most important microorganisms in the activated sludge system are the aerobic bacteria.
Bacteria

- All types of bacteria enter the wastewater treatment system.
- The floc-forming bacteria are rod shaped.
- It is the operator’s job to create an environment that will favor the floc-forming bacteria.
Bacteria
Bacteria

- Single-celled microorganisms
- Consume the biodegradable material found in wastewater
- Proteins, carbohydrates, fats and many other compounds
Bacteria

- Bacteria can only consume soluble organic material.
- Insoluble organics or particulates must be converted to soluble form before they can be consumed by the bacteria.
Bacteria: Adsorption & Absorption
Enzymes are very efficient proteins that trigger biochemical reactions in living organisms.

Under favorable conditions, they can increase the rate of biochemical reactions 10 billion times more rapidly than reactions without enzymes.
Bacteria: *Enzymes*

- Enzyme function can be altered by changes in the environment in the wastewater treatment system.
- Nutrients, temperature, pH, toxic chemicals etc. can all affect enzyme function.
Bacteria: *Enzymes*

- Without enzymes, microorganisms cannot break down organic material or perform cellular processes.
- Enzymes are strange compounds that only work when the conditions are right.
- Watch out for the “enzyme” salesman.
Bacteria: *Enzymes*

- Commercial enzymes are available for use in wastewater treatment systems.
- They can be useful when the wastewater is rich in a certain substance from a particular source.
  - Proteinase can be used to help break down protein-rich waste from meat packing industries.
Bacteria: "Super Bugs"

- Selectively or genetically engineered to do one thing better than normal bacteria.
- Genetically altered bacteria can significantly expedite the breakdown of certain substances.
  - Genetically altered bacteria are available for use in oil spills.
Bacteria: "Super Bugs"

- No super bug alone can breakdown all the substances found in wastewater.
- They are only useful when dealing with extremely difficult to treat waste products.
- Most municipal systems do not need enzyme supplements or "super bugs" under normal operating conditions.
Bacteria: *Growth Characteristics*

- Wastewater is mixed with the microorganisms in the return sludge at the beginning of the aeration basin.
- At the beginning, because an abundance of food is available, bacteria are growing, motile, and extremely active.
Bacteria: *Growth Characteristics*

- As long as enough nutrients are available, bacteria will use the nutrients mostly for growth and will multiply very rapidly by splitting into two identical cells.
- Bacteria have flagella, which enables them to swim to find available food.
Aeration Basin

Primary Effluent

RAS

TIME

FOOD
Bacteria: *Growth Characteristics*

- **Influent**
- **Return Sludge**
- **“Food”**

- **High Food**
- **Low Food**

- **Detention Time**
Bacteria: *Growth Characteristics*

- As the level of food decreases, bacteria begin to conserve energy and they lose the flagella.
- Since little food is available, they do not waste energy trying to swim to find it.
Bacteria: *Growth Characteristics*

- Next, they begin to form a thick outer slime layer.
- As they bump into each other they stick together.
- At first they form small clumps which eventually become large enough to settle.
Bacteria: Growth Characteristics

- **Floc Formation**
- **Influent**
- **“Food”**
- **Return Sludge**
- **Detention Time (Sludge Age)**

High Food vs. Low Food
Bacteria: *Growth Characteristics*

- The bacteria must remain in the aeration basin long enough to remove most of the food.
- It is only when the level of food is low, that the bacteria form the thick outer slime layer that allow them to stick together to form floc.
Bacteria

- Bacteria are structured to function very well in the activated sludge environment.
- They are natures ideal “employees”.
- They can remove most of the organic material in wastewater, multiply to meet the demand, and they know how to settle out only when the job is done.
Bacteria: Factors Affecting Performance

- Sludge Age
- Dissolved Oxygen
- Mixing
- pH
- Temperature
- Nutrients
Bacteria: **Factors Affecting Performance**

- **Sludge Age**
  - The most important key to good treatment is the formation of good floc.
  - The separation of the biological solids from treated water is important for good treatment.
Bacteria:
Factors Affecting Performance

- **Sludge Age**
  - The sludge age is how long the solids containing bacteria remain in the system.
  - Early in the process, when lots of food is available, they are very active and dispersed.
  - They have flagella, do not have a well developed slime layer, and do not settle well.
Bacteria: Factors Affecting Performance

- **Sludge Age**
  - As the sludge is allowed to age, bacteria lose their flagella and accumulate a thicker slime layer.
  - The small clumps and chains begin to stick together and form floc large enough to settle.
Bacteria: 
*Factors Affecting Performance*

- **Dissolved Oxygen**
  - Since aerobic bacteria are the primary agents in activated sludge, they require oxygen.
  - The rate of oxygen use can be correlated with the amount of available food.
Bacteria: *Factors Affecting Performance*

- **Dissolved Oxygen**
  - When the level of food is high, bacteria actively grow and use oxygen rapidly.
  - As the level of food declines, there is less activity and the requirement for oxygen decreases.
Bacteria: Factors Affecting Performance

- **Dissolved Oxygen (DO)**
  - Bacteria require 0.1 to 0.3 mg/L of oxygen to function properly.
  - Since the floc is made up of bacteria, a DO of around 2 mg/L should be maintained in the bulk fluid surrounding the floc so that sufficient DO is available for bacteria in the center of the floc.
Bacteria:
Factors Affecting Performance

- Dissolved Oxygen (DO)
  - If too little DO reaches the center of the floc, the bacteria in the center will die.
  - The floc solids will begin to break up and unfavorable bacteria that thrive under low DO conditions may begin to develop.
Bacteria: 

Factors Affecting Performance

- Mixing
  - Mixing is required to bring the bacteria, oxygen and nutrients in contact with each other
  - Without sufficient mixing, proper treatment will not take place
Bacteria:

Factors Affecting Performance

- pH
  - Enzymes are very pH dependent
  - Optimal pH is between 7.0 and 7.5
  - Rapid pH changes should be avoided
Bacteria: Factors Affecting Performance

- Temperature
  - Biochemical reactions are temperature dependent
  - Reactions are slower in colder temperatures
  - Reactions are faster in warmer temperatures
  - Therefore more bacteria are required to do the same job during the winter.
Bacteria:

*Factors Affecting Performance*

- **Nutrients**
  - Like most organisms, bacteria require nutrients to grow and maintain their cell functions.
  - They need carbon, nitrogen, phosphorus, and sulfur in addition to various metal ions such as magnesium, calcium, iron, and copper.
Bacteria: Factors Affecting Performance

- **Nutrients**
  - Most domestic wastewaters contain enough carbohydrates, proteins, and fats, which supply most of the nutrients that bacteria require.
  - It is generally industrial wastewaters that lack sufficient nutrients.
Bacteria: Factors Affecting Performance

- Nutrients
  - The ideal formula for determining the nutritional needs of microorganisms, expressed in a ratio of BOD:Nitrogen:Phosphorus (B:N:P) is 100:10:1.
  - If any of these nutrients are lacking, operational problems can result.
Bacteria:

Food : Microorganism Ratio (F:M)

F (Determined by the BOD or COD test)

M (Determined by the MLVSS or MLSS)
Food-to-Microorganism Ratio

- Changes in F:M will cause of a change or upset in the biological activity in the secondary process and may result in effluent quality problems.
F:M and Oxygen Uptake

- Actively growing bacteria consume at a rapid rate utilizing oxygen at a rapid rate.
- Generally a higher Uptake Rate is associated with a higher F:M ratio and younger sludge ages.
- A lower Uptake Rate is associated with a lower F:M ratio and older sludge ages.
Protozoa

- Bacteria make up 95% of microorganisms in activated sludge and are primarily responsible for removing organic material.
- About 4% are protozoa.
- Protozoa do play a critical role in the treatment process by removing and digesting free-swimming dispersed bacteria and other suspended particles.
PROTOZOA

- Most protozoa are aerobic microorganisms
- Some smaller protozoa take in soluble nutrients through the cell membrane
- Others have specialized structures or mouth-like openings and feed on other microorganisms such as bacteria and algae and other solid matter
Protozoa

- Protozoa are classified based on how they move:
  - Amoeba
  - Flagellates
  - Ciliates
    - Free-swimming ciliates
    - Crawling (grazing) ciliates
    - Stalked (sessile) ciliates
Protozoa

- The wastewater operator need not know how to precisely identify all of the species of protozoa.
- It is helpful to classify them into one of the five categories.
Aeration Basin

Primary Effluent

RAS

FOOD

BACTERIA
Protozoa: *Amoeba*

- The most primitive single celled protozoa.
- They feed on small organic particulates, but will eat algae, bacteria and other small protozoa.
- They are more drawn to particulate matter.
Protozoa: *Amoeba*

- Amoeba contribute very little to the overall treatment of wastewater.
- They move very slowly and cannot compete with bacteria or other protozoa for food.
- They can only dominate early in the treatment process when the food level is quite high.
Protozoa: *Amoeba*

- Moves slowly by extending lobe-like projections called pseudopods until they have enveloped the food to form a vacuole

- Enzymes secreted into the vacuole break down food to be absorbed into the cell
Protozoa: *Amoeba*
Protozoa: *Amoeba*
Protozoa: *Amoeba*
Protozoa: *Amoeba*
Protozoa: Amoeba
Protozoa: *Amoeba*

- The presence of large numbers of amoeba in the mixed liquor sample indicate:
  - Shock loading of BOD
  - The presence of large amounts of particulate matter
  - Lack of oxygen
  - Low levels of toxicity or other unfavorable conditions
Protozoa: *Flagellates*

- Most flagellates absorb nutrients just like bacteria
- Flagellates compete with bacteria for dissolved nutrients
- Flagellates peak in number while the soluble food concentration is high
Protozoa: *Flagellates*

- Variety of sizes and shapes
- Easily distinguished by its whip-like structure called a flagellum
- The flagellum is used for locomotion and feeding
Protozoa: Flagellates
Protozoa: *Flagellates*
Protozoa: **Flagellates**

- The presence of large numbers of flagellates in the mixed liquor sample indicate:
  - Incomplete treatment
  - Shock loading of BOD
  - The presence of large amounts dead or decaying material
Protozoa: *Ciliates*

- For the purpose of studying activated sludge, ciliates will be classified based on their ability to compete for food
  - Free-swimming ciliates
  - Crawling (grazing) ciliates
  - Sessile (stalked or attached) ciliates
Protozoa: *Free Swimming Ciliates*

- Appear as the flagellates begin to disappear
- As the bacteria population increases there is a lot of disperse bacteria available for feeding
- Free-swimming ciliates begin to dominate as they feed on the increased number of bacteria
Protozoa: *Free-swimming Ciliates*

- Generally uniformly covered with cilia which is used for locomotion
- Move quickly through the water by swimming or spiraling
- Free, dispersed bacteria are their principal food
Protozoa: *Free Swimming Ciliates*
Protozoa: *Free Swimming Ciliates*
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Protozoa: *Free Swimming Ciliates*
Protozoa: *Free Swimming Ciliates*

- The presence of large numbers of free-swimming ciliates indicate:
  - An abundance of active bacteria
  - Early in the treatment process
  - Nutrients have not been depleted
Protozoa: *Crawling Ciliates*

- As floc particles enlarge, crawling ciliates graze on floc particles.
- As the population of disperse bacteria decreases and floc increases, crawling ciliates outcompete free-swimming ciliates because they can find food within the floc.
Protozoa: *Crawling Ciliates*

- Crawlers have cilia that are joined together to form tufts or legs (cirri).
- Instead of swimming the cirri function as coordinated legs that allow them to walk on surfaces.
- They crawl along floc particles and pick at any straggling bacteria.
Protozoa: *Crawling Ciliates*
Protozoa: *Crawling Ciliates*
Protozoa: *Crawling Ciliates*
Protozoa: *Crawling Ciliates*

- The presence of large numbers of crawling ciliates indicate:
  - Most of the organic material has been removed
  - Bacteria are clumping together to form floc
  - Adequate detention time
Protozoa: *Sessile Ciliates*

- Two major types of Sessile Ciliates
  - Stalked
  - Attached w/o a stalk
Aeration Basin

Primary Effluent

RAS

Daniel

Lawrence
Sessile Ciliates: *Stalked*

- Feed mostly on suspended bacteria, algae, or smaller protozoa.
- Rarely swim freely but can be found attached to almost anything.
- The cilia are used to create a current that bring food particles into the mouth.
Sessile Ciliates: *Stalked*

- Some grow from single stalks while others can branch into colonies of more than 100.
- Some stalks contain a central muscle fiber (myoneme) that caused the ciliate to contract when disturbed.
Sessile Ciliates: *Stalked*

*Vorticella*
Sessile Ciliates: *Stalked*  

*Vorticella*
Sessile Ciliates: *Stalked*
Daniel in Action
Daniel and Mom Racing for Food
Sessile Ciliates: \textit{Stalked}

\textit{Epistylis}
Sessile Ciliates: *Stalked* 

*Carchesium*
Sessile Ciliates: *Stalked*
Sessile Ciliates: *Stalked*
Sessile Ciliates: *Stalked*

*Opercularia*
Sessile Ciliates: *Stalked*
Sessile Ciliates: *Stalked*

- The presence of large numbers of stalked ciliates indicate:
  - Most of the organic material has been removed
  - As sludge ages, the dominance of stalked ciliates changes from single stalks to colonial species
  - “The greater the number of heads, the older the sludge”
Sessile Ciliates

- As the sludge begins to age, the more dominant types of sessile ciliates will begin to dominate
  - Stentor
  - Suctorian
Aeration Basin

Primary Effluent

RAS

Rubin Jr.
Sessile Ciliates

Stentor
Sessile Ciliates
Sessile Ciliates
Protozoa: *Attached Ciliates*

*Stentor*
Protozoa: *Attached Ciliates*

*Stentor*
Protozoa: Attached Ciliates
Aeration Basin

Primary Effluent

RAS

Ex-Mother-in-law
Sessile Ciliates

Suctoria
Sessile Ciliates
Protozoa: *Attached Ciliates*

*Suctoria*
Protozoa: Attached Ciliates

*Suctoria*
Protozoa: 

*Factors Affecting Protozoa*

- Temperature
- pH
- Dissolved Oxygen
- Nutrients
Protozoa: Factors Affecting Protozoa

- Temperature
  - Their metabolism is slower in colder temperatures.
  - Their metabolism doubles for every 10 degree rise in temperature.
  - The grow best at ambient temperatures around 15 to 25 degrees C.
Protozoa:  
*Factors Affecting Protozoa*

- **pH**
  - Protozoa are more sensitive to pH than floc-forming bacteria are.
  - They have an optimum range of 7.2 - 7.4 but can tolerate 6.0 - 6.8
Protozoa: Factors Affecting Protozoa

- Dissolved Oxygen
  - Like bacteria, protozoa must have oxygen to survive.
  - Lack of oxygen will severely limit the kind and number of protozoa present.
Protozoa: Factors Affecting Protozoa

- **Nutrients**
  - Most municipal wastewater systems contain sufficient nutrients to support most protozoa.
  - Industrial wastes are more likely to be deficient in nutrients.
METAZOA

- Metazoa include all multicellular organisms including microorganisms.
- Metazoa have very little to do with the removal of organic material from the wastewater.
- Metazoa dominate in longer age systems including lagoon treatment systems.
Metazoa

- Multi-cellular microorganisms that feed on bacteria, algae and protozoa.
  - Rotifers
  - Nematodes
  - Gastrotrich
  - Bristle worms
  - Tartigrades (water bear)
  - Ostracods (Daphnia)
Metazoa: *Rotifers*

- Their principal contribution is to clarify the effluent by removing leftover bacteria, algae or other smaller protozoa.
- Sometimes as many as 4 types of rotifer can be present in the system at one time.
- Rotifers should never dominate in the treatment system.
Metazoa: Rotifers
Metazoa: *Rotifers*
Metazoa: *Nematodes*

- Come in various sizes
- Move through the fluid by wiggling back and forth
- Feed on bacteria, fungi, small protozoa and sometimes other small nematodes
- Some species have teeth and a spear that they can stick into their prey
- The spear is used as a straw to suck food
Metazoa: *Nematodes*
Metazoa: *Nematodes*
Metazoa: *Gastrotrich*

- Colorless worm; relative of the Nematode
- Feed on living or dead organic material.
- Some species of Gastrotrichs have the shortest life span of all the Metazoa; only 3 days.
Metazoa: *Gastrotrich*
Metazoa: *Tartigrade (Waterbear)*

- Commonly found in the same environment as rotifers and nematodes
- They feed on algae and small protozoa
- They can survive extreme environmental swings
- They are very sensitive to toxic conditions
- They do not survive well in the presence of ammonia
Metazoa: Waterbear
Metazoa: Waterbear
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Metazoa: Waterbear
Metazoa: Waterbear
Metazoa: Waterbear
Metazoa: Waterbear
Metazoa: *Annelid (Bristleworm)*
Metazoa: Annelid (Bristleworm)
Metazoa:

Annelid (Bristleworm)
Metazoa: *Ostracod (Daphnia)*
Process Control: Microscopic Observations

- Dispersed Growth
- Nutrient Deficiency
- Slime Bulking (India Ink Stain)
- Protozoa Count
- Toxicity/Adverse Affects
The primary role of bacteria

- Removal of BOD
- Produce more bacteria
- Form biological floc large enough and compact enough to settle.
Process Control: *Dispersed Bacteria*

- A population of suspended, growing, non-flocculated bacteria, algae or fungi (most is bacteria)
- In a properly operating system little dispersed growth should be present in the liquid around the floc.
Dispersed Bacteria
Process Control: *Dispersed Bacteria*

- Dispersed bacteria is removed from the liquid as bacteria develop the "slime" layer and clump together to form floc.
- Dispersed bacteria is also removed by ciliates and rotifers.
Process Control: *Dispersed Bacteria*

- The presence of significant dispersed bacteria is due to improper floc formation
- Factors for improper floc formation:
  - young sludge
  - toxicity
  - slug discharge
  - lack of ciliates
  - excessive shearing and/or surfactants
Process Control: *Dispersed Growth*
Process Control: *Dispersed Growth*
Process Control: *Dispersed Growth*
Nutrient Deficiency

- Often in industrial and municipal system nutrient deficiency may occur
- Nitrogen and Phosphorus are the nutrients that are usually deficient.
- Nutrient ratio 100:10:1 (BOD:N:P)
BACTERIA CELL

Cross Section
Nutrient Deficiency

**Gram positive**
- Polysaccharide
  - “Slime Layer”
- Cell Wall
- Cell Membrane

**Gram negative**
- Lipopolysaccharide
  - “Slime Layer”
- Phospholipid
- Lipoprotein
- Cell Wall
- Cell Membrane
Nutrient Deficiency

- Nitrogen is required for the development of the lipoprotein layer.

- Phosphorus is required for the development of the phospholipid layer.
Nutrient Deficiency

- When Phosphorus is deficient, a small amount of extra lipids (fat) is added to the slime layer.
Nutrient Deficiency

- When Nitrogen is deficient a larger amount of “fat” is added to the slime layer.
Microscopic Observations: *Nutrient Deficiency*
Microscopic Observations: *Nutrient Deficiency*
Microscopic Observations: 
Nutrient Deficiency
Microscopic Observations:

*Nutrient Deficiency*
Microscopic Observations: *Nutrient Deficiency*
Process Control: *Slime Bulking*

- Nutrient Deficiency will result in:
  - nutrient deficient floc particles (the production of an exocellular polymer-like substance)
  - loss of settleability (slime bulking)
  - possibility of foaming (slime foaming)
Nutrient Deficiency: Slime Bulking
Nutrient Deficiency: Slime Bulking

NORMAL

NUTRIENT DEFICIENT
Process Control: *Slime Bulking*

- India Ink stain
- When India ink is added to a drop of mixed liquor the carbon black particles penetrate the floc from outside to inside
- The exocellular polymer-like substance prevents the India ink from penetrating nutrient deficient floc.
Process Control: *Slime Bulking*
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Process Control: *Slime Bulking*

- Operational Considerations
  - The solution involves adding the deficient nutrient
  - Ammonia to provide nitrogen
  - Phosphoric acid to provide phosphorus
Process Control: *Slime Bulking*

- Operational Considerations
  - There is no nutrient deficiency if, in a filtered (0.45 µm) effluent sample:
    - ammonia + nitrate is > 1 mg/L and,
    - soluble orthophosphate is > 0.5 mg/L
Process Control: *Slime Bulking*

- **Excess Organic Acids**
  - A ready food source that does not contain nitrogen
  - Usually added through sludge processing recycle streams (anaerobic digester supernatant)
  - Any other anaerobic process side stream
Process Control: *Slime Bulking*

- The excess lipopolysaccharide can be wasted out of the system or eaten by the bacteria.
- You have to stop the bacteria from producing the excess amounts.
  - Making sure sufficient nutrients are available
  - Adding anaerobic recycle streams slowly and/or add more organisms (increase return) when excessive amounts of organic acids are present.
Process Control: *Protozoa Count*

- Relative Abundance
- Dominant Groups
- Recessive Groups
Process Control: *Protozoa Count*

- **Relative Abundance**
  - The relative number of protozoa in each of the protozoa groups
    - Amoeba
    - Flagellates
    - Free-swimming ciliates
    - Crawling ciliates
    - Stalked ciliates
    - Metazoa (rotifers, nematodes, waterbear)
    - Shelled species
Process Control: *Protozoa Count*

- The 3 groups with the highest percentages are the *dominant groups*.
- In a well operating system the three dominant groups should be:
  - free-swimming ciliates
  - crawling ciliates
  - stalked ciliates
Process Control: *Protozoa Count*

- The two protozoa groups with the lowest percentages are the *recessive groups*.
- In a well operating system the recessive groups should be:
  - Amoeba
  - Flagellates.
Process Control: *Protozoa Count*

- Use a well-mixed, representative sample, dilute if necessary.
- Use a wet-mount procedure and scan at 20x or 40x using either phase or bright-field.
- Scan the slide in 5 passes, be consistent in the number of passes.
- Count and record the number of each of the types of microorganisms you see.
Process Control: *Protozoa Count*
Process Control: *Protozoa Count*

- Step 1 - Calculate the relative abundance
  - calculate the total count per milliliter for each of the types
  - \( \# \text{ protozoa/mL} = \# \text{ protozoa} \times 20 \)
  - Take the counts from 3 to 5 slides and average the numbers for each group and use this number to calculate relative abundance
Process Control: *Protozoa Count*

- Step 2- Determine dominant and recessive groups
- Calculate the percent for the total of each of the groups

\[
\frac{\text{# protozoa/mL in the group}}{\text{Total number of protozoa counted}} \times 100
\]
Under adverse or unfavorable conditions, bacteria, protozoa and metazoa develop a variety of protective mechanisms to shield themselves from the harsh environment.
Toxic/Adverse Affects - *Bacteria*

- Under toxic or other adverse conditions bacteria take steps to protect themselves
  - Some bacteria surround themselves with protective encasements
  - More obviously in activated sludge bacteria will develop zoogleal communities
What is Zooglea?

- Zooglea is a colony of microbes embedded in a polymeric matrix.
What is Zoolea?

- The term “zoolea” is often confused with the bacteria species *Zooglea ramigera*.
- There are several different types of bacteria that have the ability to form zooleal communities and *Z. ramigera* is only one of them.
What is Zooglea?

- There are basically two types of bacteria in the activated sludge system; those that can form floc and those that cannot.
- Bacteria that form floc have very specific characteristics.
Why is zoogelea formed?

- Zoogelea protects the microbial community from environmental stresses.
  - Toxicity
  - pH shifts
  - Osmotic shock
  - Dessication
  - Excess organic acids
Toxic/Adverse Affects - Bacteria

- Bacteria distribute themselves geographically within the zoogoleal structure based on who their neighbors are and the environment that best suits their needs and requirements.

- They are not randomly distributed but organized to best meet the needs of each.
Zoogleal Formations
Zoogleal Formations
Zoogleal Formations
Zoogleal Formations
Zoogleal Formations
Zoogleal Formations
Toxic/Adverse Affects - *Bacteria*

Zoogleal Formation

- Conditions that affect bacteria also affect the protozoa
- It is difficult for protozoa to feed on bacteria that are surrounded and protected by zoooglea.
Toxic/Adverse Affects - *Bacteria*

Zoogleal Formation
Toxic/Adverse Affects - *Bacteria*

Zoogleal Formation
Toxic/Adverse Affects - *Bacteria*

Zoogleal Formation
Toxic/Adverse Affects - *Bacteria*

Zoogleal Formation
Protozoa have a variety of mechanisms for protections against unfavorable conditions.

- Some form shells, some form tubes and others will detach and search for more favorable conditions.
Toxic/Adverse Affects - *Amoebae*

- Under adverse conditions amoebae that have the ability to form shells will begin to dominate.
- Some amoeba will form shell by collecting inert material from the surrounding fluid while others secrete substances from within to form the shell.
Protozoa: \textit{Testate Amoeba}
Protozoa: *Testate Amoeba*

*Arcella*
Protozoa: *Testate Amoeba*
Protozoa: *Testate Amoeba*
Protozoa: *Testate Amoeba*
Protozoa: *Testate Amoeba*
Protozoa: *Testate Amoeba*
Protozoa: Testate Amoeba

Euglypha
Protozoa: *Testate Amoeba*
Protozoa: Testate Amoeba
Protozoa: *Testate Amoeba*
Toxic/Adverse Affects - *Flagellates*

- Under adverse conditions flagellates will create a gelatinous matrix to dwell in.
- Some flagellates that form “collars” are better suited for adverse conditions.
Protozoa: *Flagellates*
Protozoa: *Flagellates*
Toxic/Adverse Affects – *Stalked Ciliates*

- Under adverse conditions stalked ciliates will dislodge from their stalk.
- The “head” or zooid will swim in search for a more favorable environment.
- If a more favorable environment is found it will stay and grow another stalk.
Protozoa: Stalked Ciliates
Toxic/Adverse Affects – *Stalked Ciliates*
Toxic/Adverse Affects – *Attached Ciliates*

- Under adverse conditions attached ciliates that have the ability to form protective “tubes” will begin to dominate.
- The “tube-dwellers” will remain within the tube until conditions become favorable.
Protozoa: Tube Dwellers
Protozoa: *Tube Dwellers*
Protozoa: *Tube Dwellers*
Protozoa: *Tube Dwellers*
Toxic/Adverse Affects – *Attached Ciliates*

- Under adverse conditions attached ciliates that have the ability to form protective “tubes” will begin to dominate.
- The “tube-dwellers” will remain within the tube until conditions become favorable.
- Once conditions become favorable they will abandon the tube.
Toxic/Adverse Affects – Attached Ciliates
Toxic/Adverse Affects – Rotifers

- Under adverse conditions some rotifers will form shells that resemble a turtle shell.
- Others form tubes or gelatinous capsules.
Metazoa: *Shelled Rotifers*
Metazoa: *Shelled Rotifers*
Metazoa: *Shelled Rotifer*
Metazoa: *Shelled Rotifer*
Metazoa: Shelled Rotifers
Rotifer: *Tube Dwellers*
Water Bear: *Cryptobiosis*

- *Cryptobiosis* – A process by which the water bear essentially stops all metabolic activity.
- It will turn itself into a husk or spore of its former self that will reawaken when conditions are right.
- You just add water and it comes back to life, just like sea monkeys.
Process Control: *Toxicity*

- Other microscopic indications of toxicity include:
  - Loss of higher life forms
  - Poor floc formation (disperse growth)
  - Unusually low oxygen use
  - Poor BOD removal
  - Flagellate bloom
  - Filamentous bulking upon recovery
Most Important

- Keep a process chart of treatment system parameters
- Measure the parameters routinely and consistently
- Measure the parameters when the treatment system is working properly
“Well, yes, we could fix it in Photoshop, but your arm would still be broken.”