

Activated Sludge Microbiology and Microbial Ecology

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Thoughts

- What are the critters?
- How do they do what they do?
- What do they do for us?
 - Feed on Biochemical Oxygen Demanding food
 - Process BOD, NH_3 , etc. - focus of most people
 - “SETTLE!!”
- How do they relate to each other?
- How do we control them?

The Process

- Earl B. Phelps, USPHS, 1917, (three years after the Arden and Lockett experiments): “The accepted current use of the word **process** to define the engineering **apparatus**, such as the trickling filter process, etc., while convenient ..., frequently tends to obscure the essential **bio-chemical** nature of the treatment...”
- It’s the microorganisms that are the process!!

Different Types of Critters

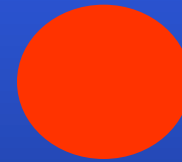
- Bacteria – do most of our treatment work
- Archaea – old, old, old – first critters?
 - Tend to like extreme environments, e.g., low DO, methane formers, Yellowstone hot springs
 - Don't know much about them yet
- Protozoa - just fun to watch in a microscope
 - Clean-up the water
- Metazoa
 - Big, ugly, clean up, eat the other guys

Most Important Bacterial/Archaeal Cell Shapes

- Rod Shaped
(bacillus)



- Spherical (coccus)



0.5-3 μ balloons with thousands of holes -
Tiny mouths and waste-discharge ports
Feed very fast on soluble BOD -
example - sugar

Bacillus (Rod Shape)



Single Cells



Pairs



Floc

Basic shape

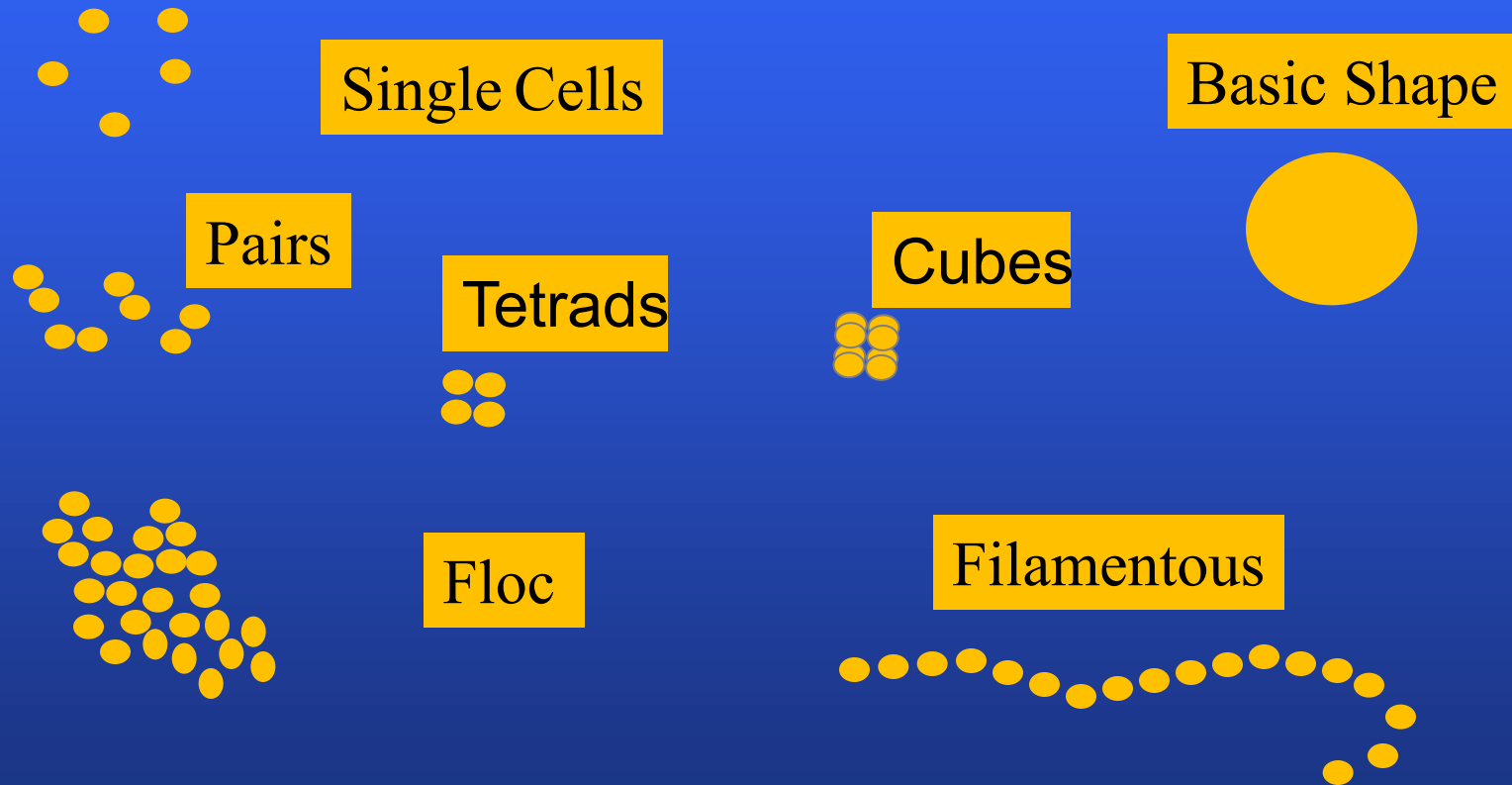


Filamentous



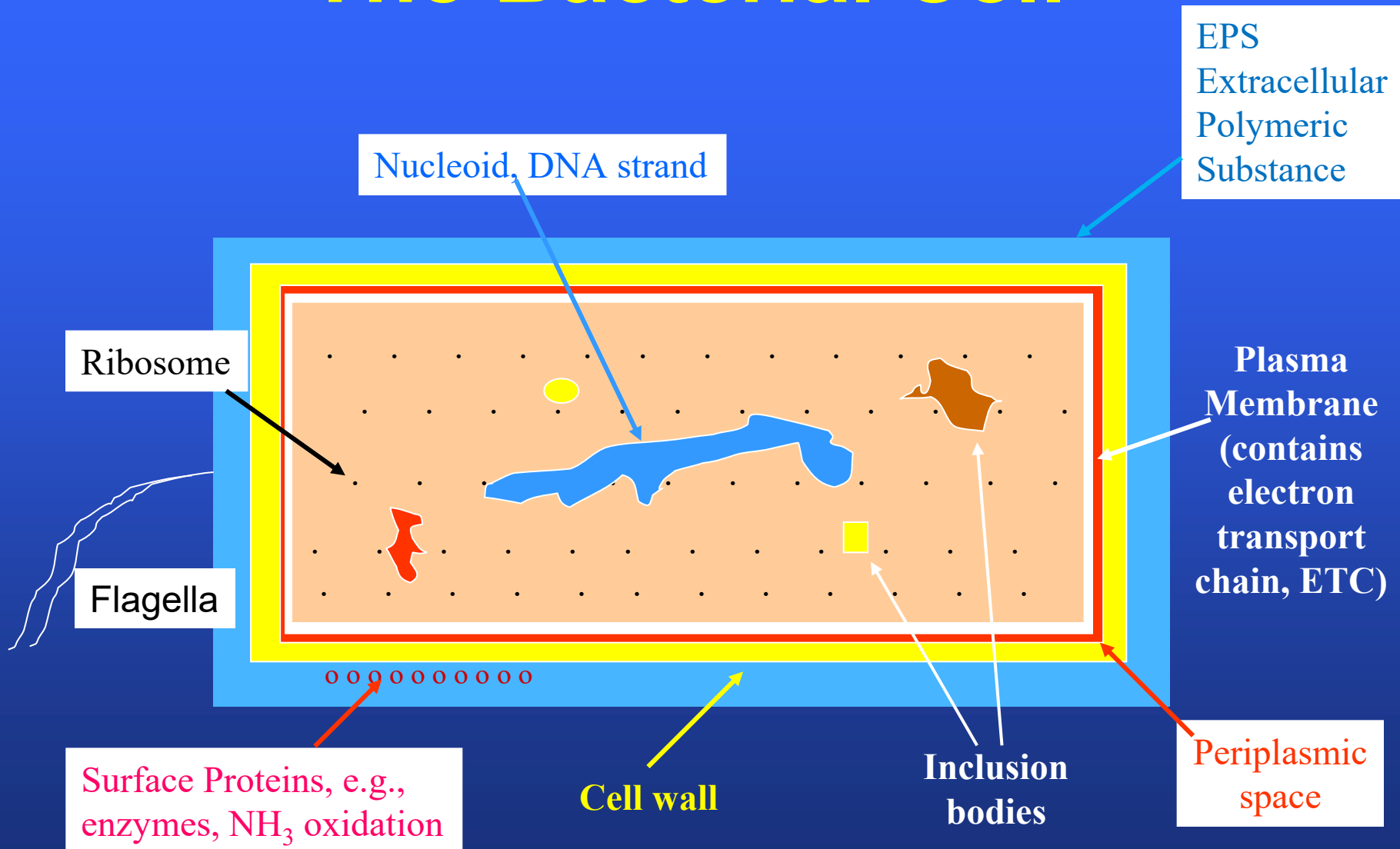
More common in low organic concentrations (ZoBell 1946)
e.g., activated sludge

Coccus (Round Shape)

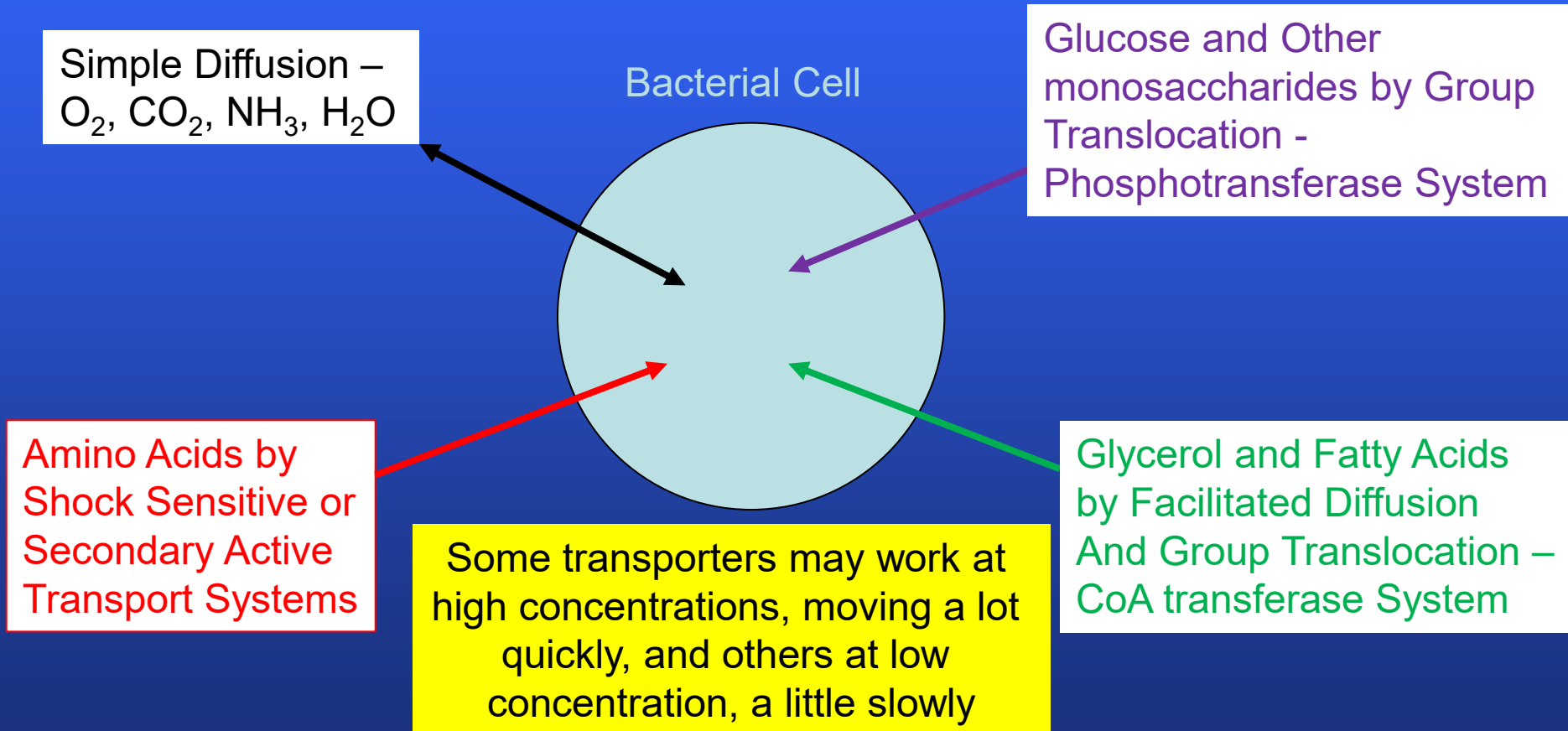


More common in nutrient-rich environments – ZoBell 1946

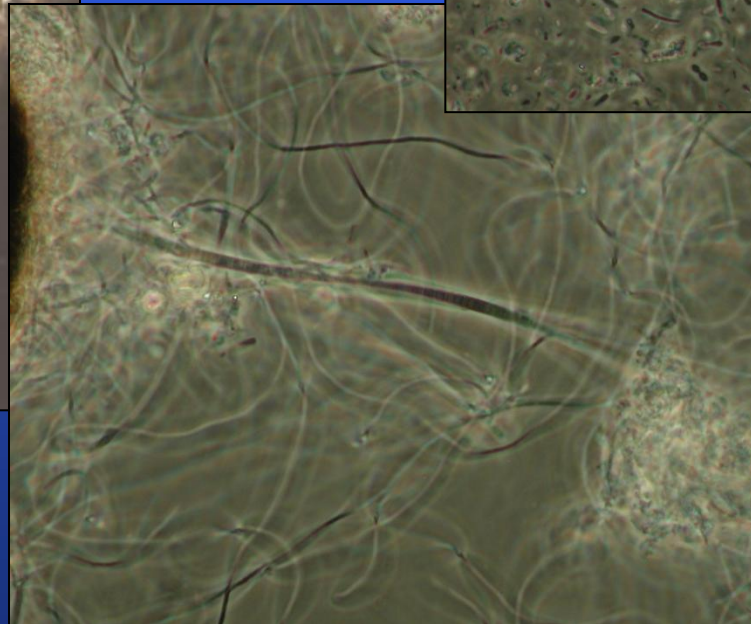
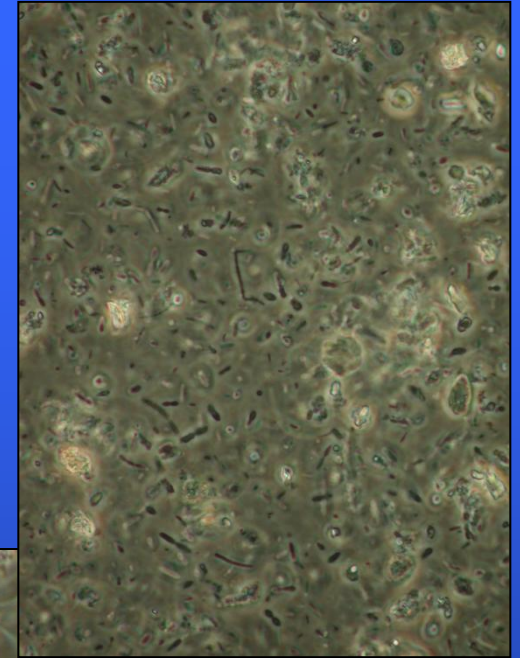
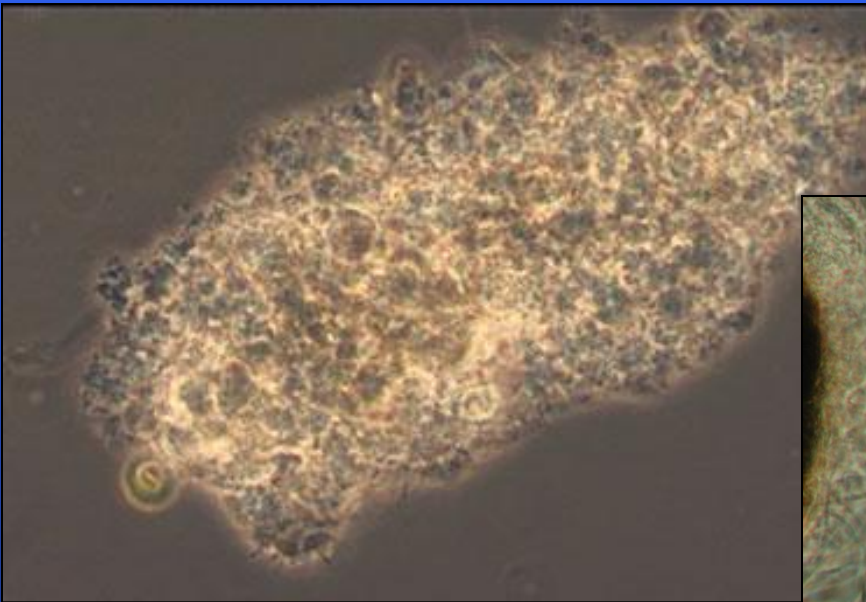
The Bacterial Cell



Absorption of Soluble BOD and Other Nutrients Thru Transporters (holes)



Flocs, Filaments & Dispersed Growth



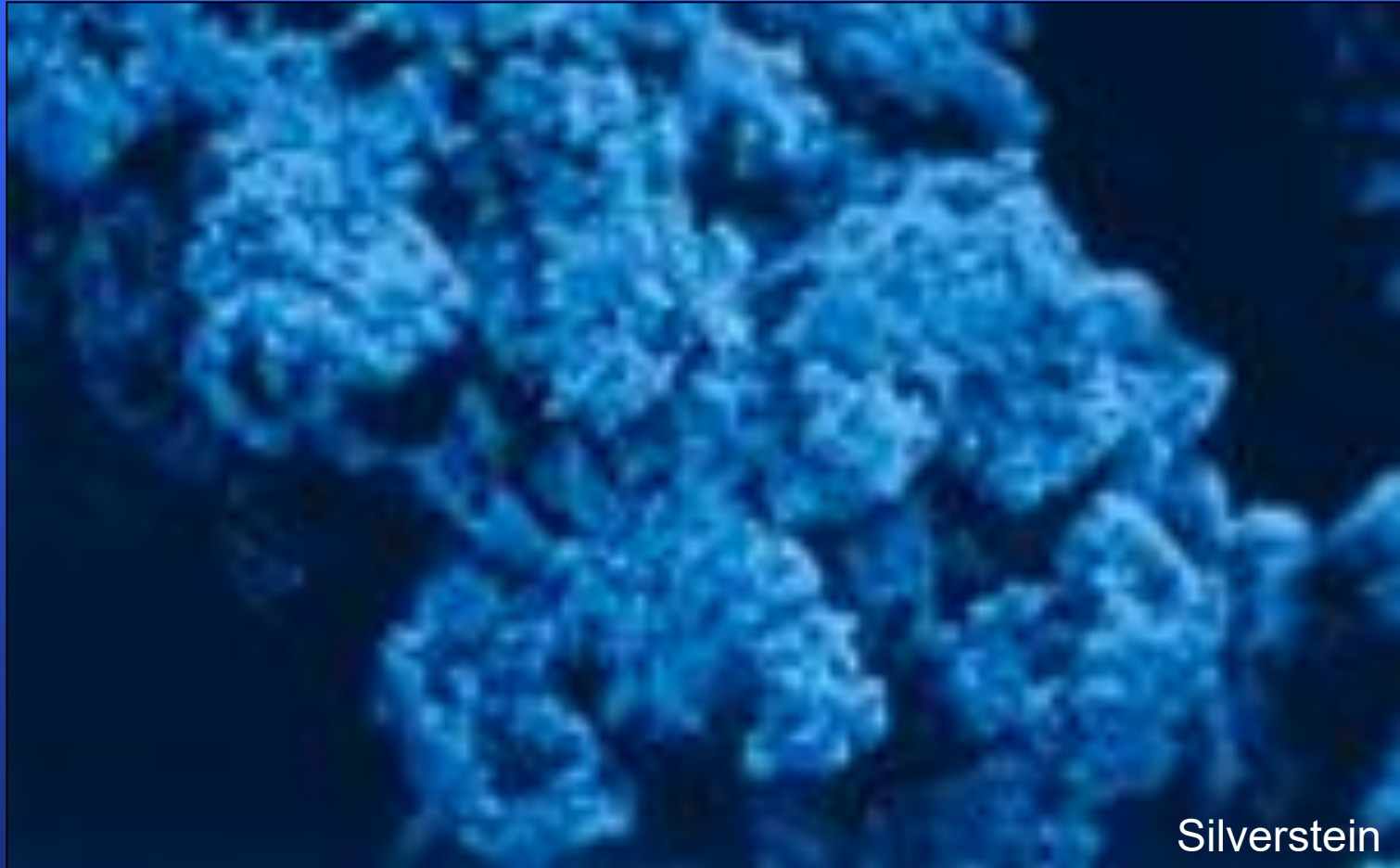
Floc-Forming Bacterial Genera

- *Pseudomonas*
- *Flavobacterium*
- *Achromobacter*
- *Bacillus*
- *Alcaligenes*
- *Micrococcus*

■ *Floc characteristics*

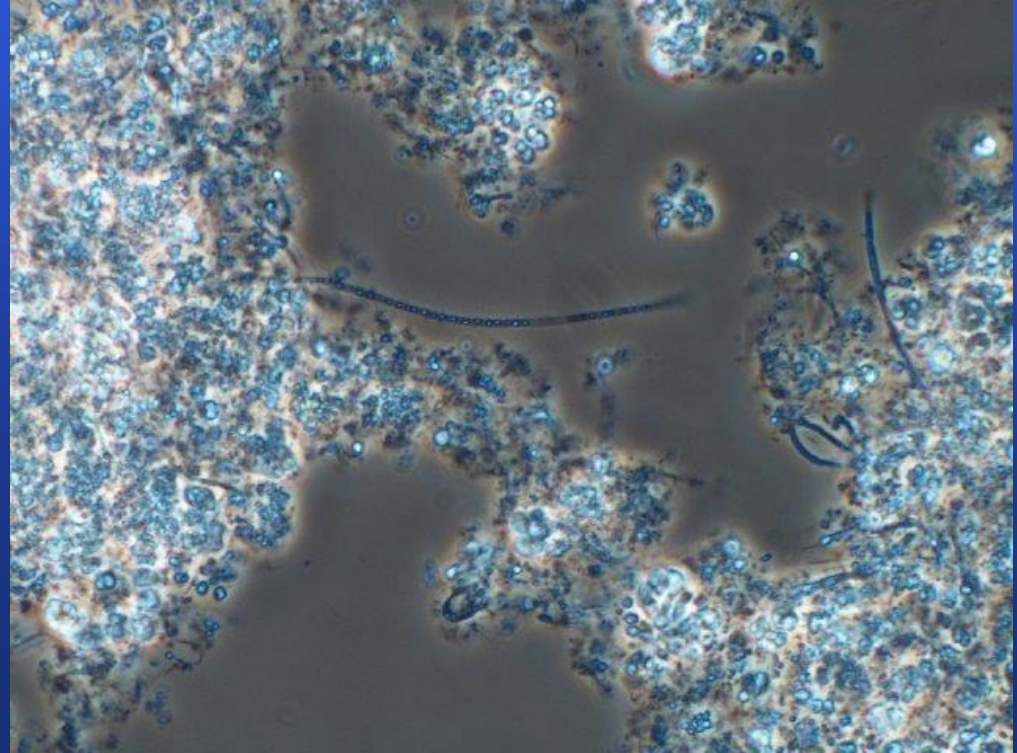
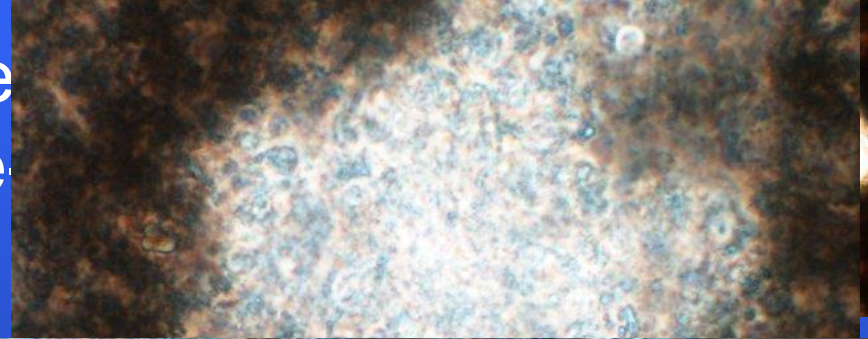
- *Dense* (1.03 - 1.056 g/mL)
- *Rounded*
- *Bio-P Sludges typically heavier*
 - *Storage products*
 - *Poly-P (1.23), glycogen (1.25), PHB (1.15)*

Electron Photo of Floc-Former Floc



Excess Polysaccharide

- Floc-formers may produce polysaccharide (slime) in high available BOD/low nutrient conditions
 - N & P
 - Micronutrients
 - Fe, Co, Mo, Cu, Zn
 - And others
- Slime is stored BOD



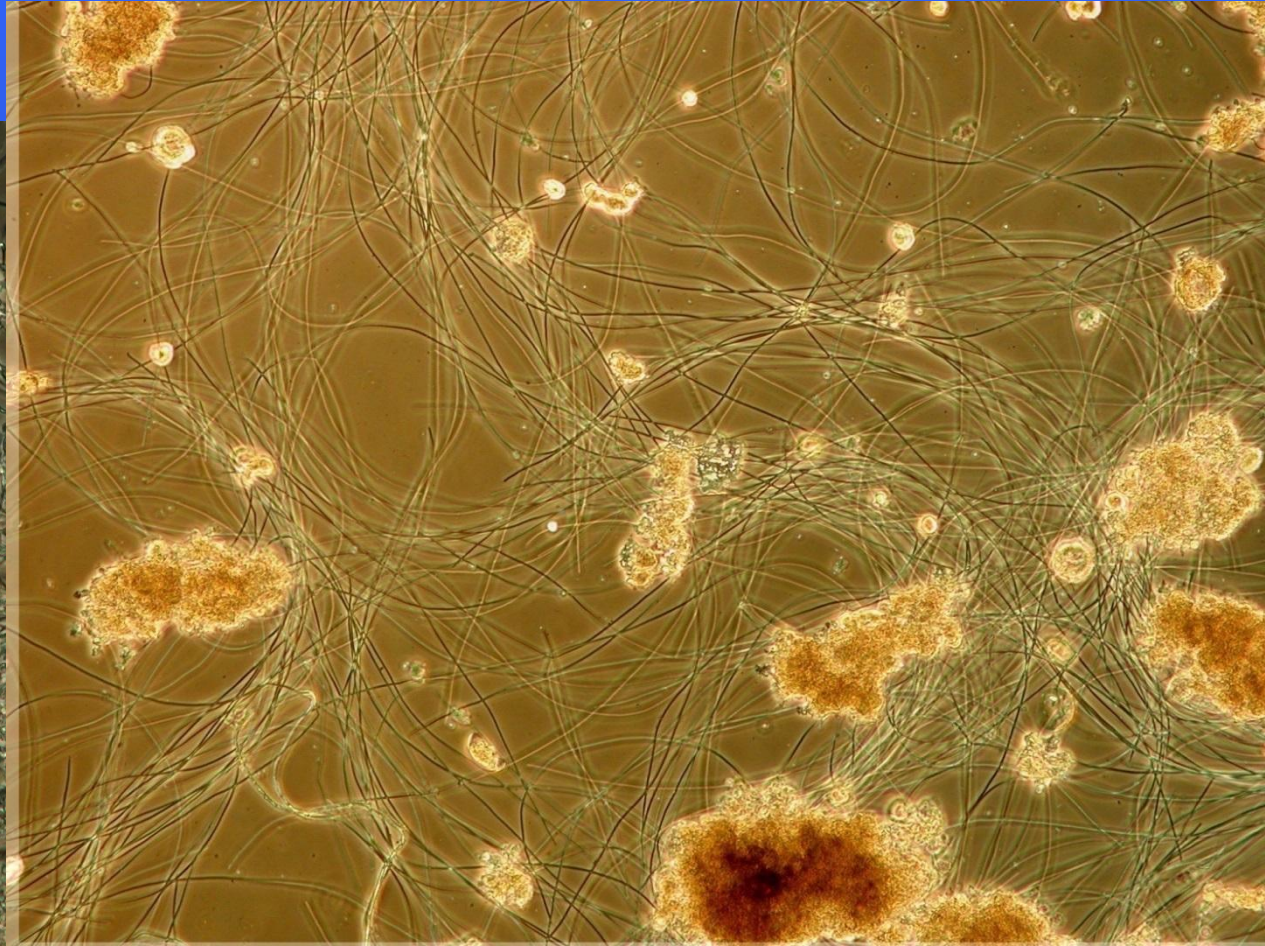
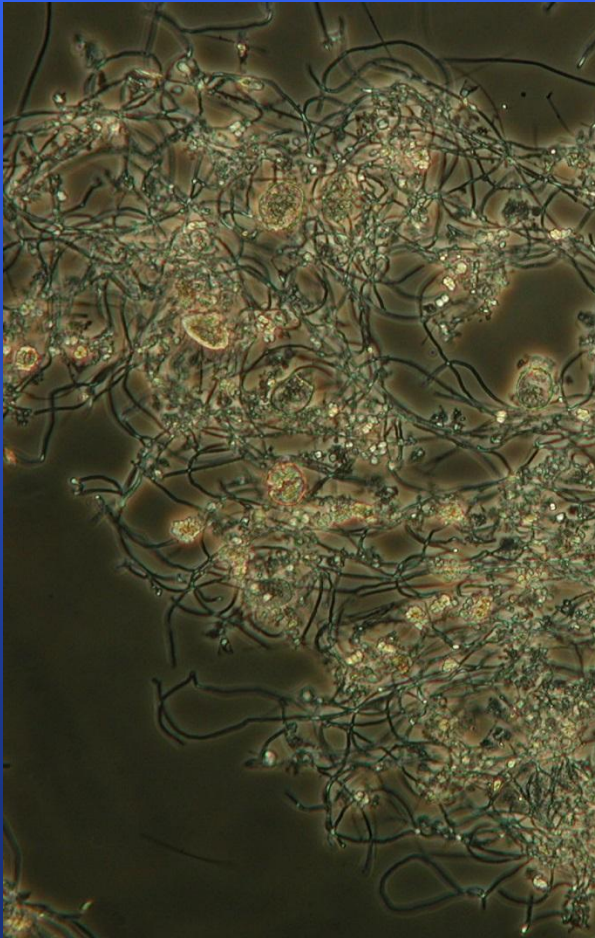
Electron Photo of Filamentous, Open Floc & the Sponge Effect



Excessive Filament Growth

Between the Flocs

Inside the Floc



Common Filamentous Species

Many yet to be identified - DNA

- *Beggiatoa*
- *Haliscomenobacter hydrossis*
- *Microthrix parvicella*
- *Nocardioforms** – many genera
- *Nostocoida limicola** I, II & III
 - I – *Tricococcus* spp.
 - II – New genus, various proposed
 - III – *Isophaera* spp.
- *Sphaerotilus natans*
- *Thiothrix* I, II, type 021N
- Probably well over 100 species

* These are being modified as more genetic information becomes available. Rather than identifying species, it is better to list the group to which the filament belongs, e.g., low-DO, septicity, low F/M, septicity, nutrient deficiency, or low pH,

Common Eikelboom Types

- 0041
- 0092
- 021N*
 - *Several Thiothrix spp.*
- 0675
- 0914
- 0961
- 1701*
 - *Sphaerotilus spp.*
- 1851
- 1863*
 - *Acinetobacter* or *Moraxella*

- Floc characteristics
 - Irregular shape, possible growth beyond floc edge
 - Low density (1.02 - 1.03 g/mL)

Filament Growth Environments

- DO - low
- Septicity → Organic Acids (>100 mg/L) & S²⁻
- Organic loading rate and biodegradability
 - Low F/M (low food load per bug)
 - Slowly degradable materials (hard to digest)
- pH - low
- Nutrient deficiency (N, P, micronutrients)
- Completely mixed, continuously fed
 - Just a little bit - all the time

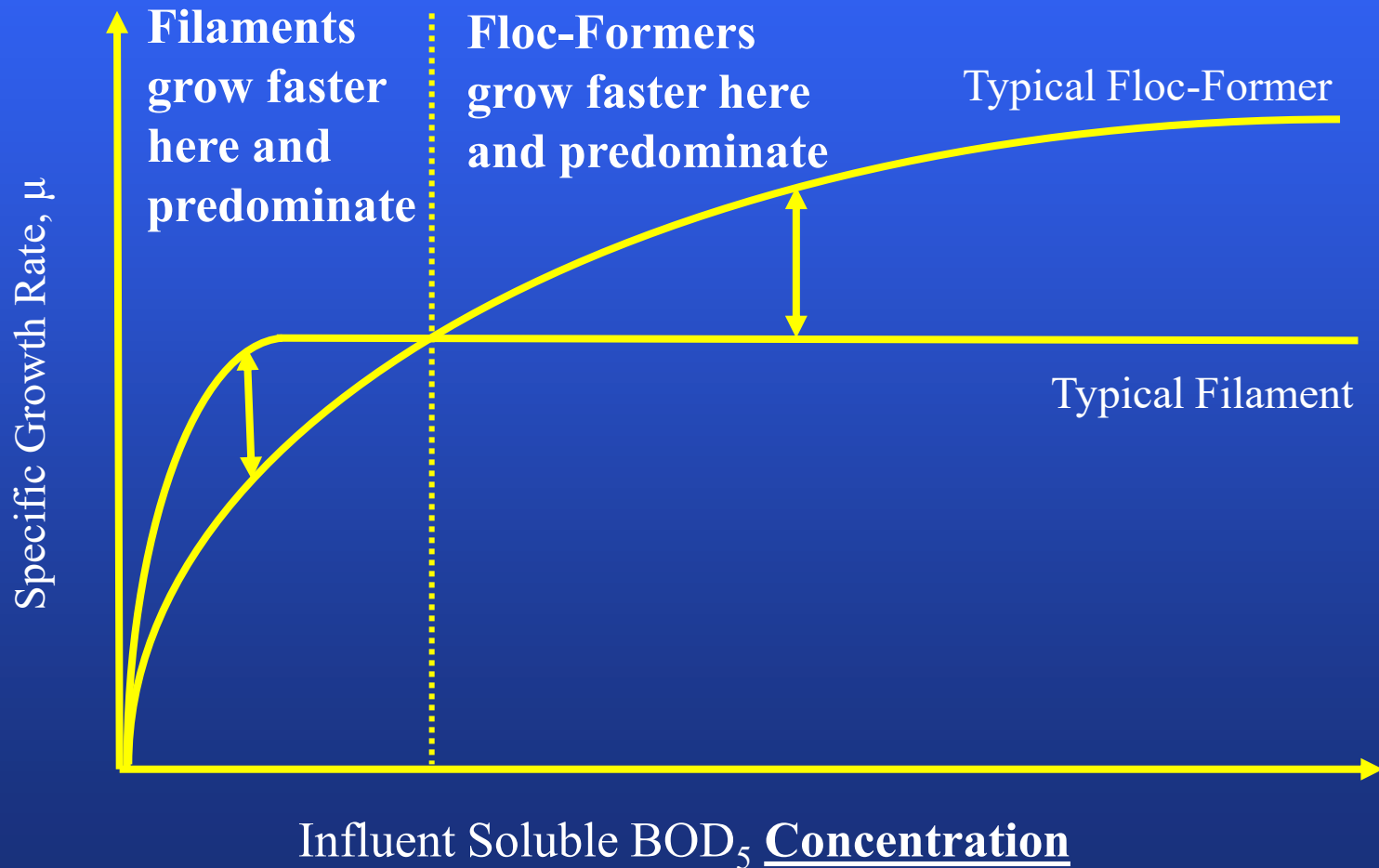
Filament Growth

- Filaments typically take advantage of low concentrations
- Long, narrow shape provides large surface to volume ratio
 - More surface to adsorb and absorb materials
- Which has the best contact with low concentration of food, DO, or ????



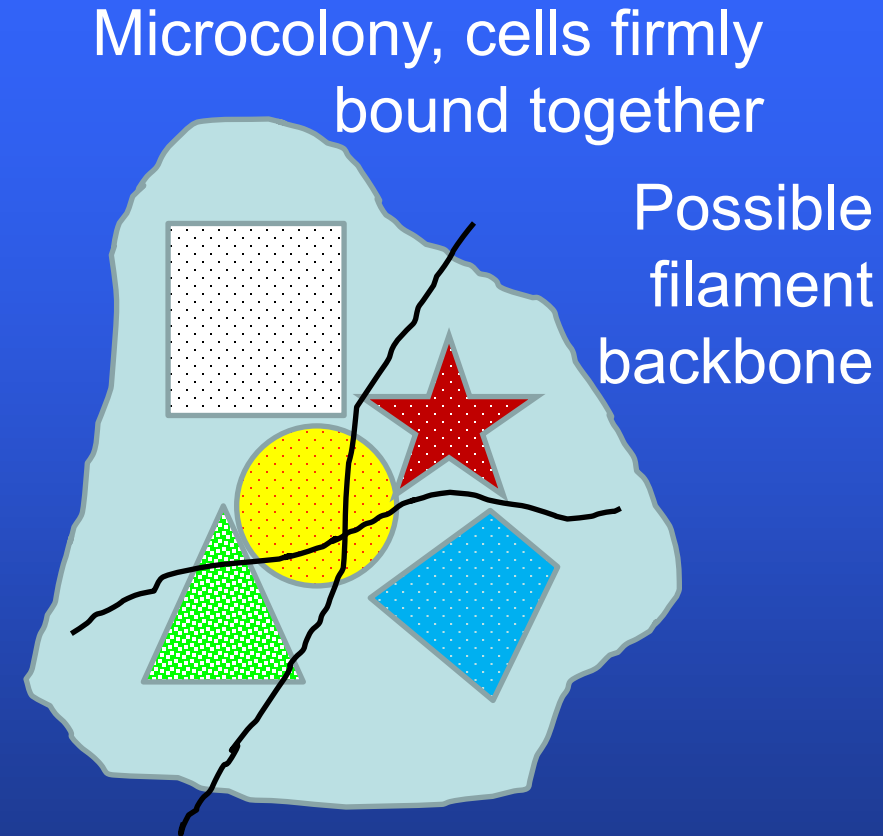
Filaments

Floc vs. Filament Growth Rate



Floc Formation - EPS

- EPS
 - Extracellular polymeric substances
- Poor flocculation appears due to loosely attached EPS inability to hold microcolonies together
- Held together
 - Van der Waal's
 - Electrostatic
 - Multi-valent cations
 - Hydrophobic interactions
 - Polymer entanglements



Microcolonies loosely held together in common gel matrix

Ecology

- The study of the relationships of organisms to their environment
- Ecological problem orientation
 - Habitat – chemical, biological, physical
 - Organism – which organisms
- Our problem approach looks at both habitat and organism

Already Familiar with Ecology

- Big guys
 - Hunter – elk or deer
- Smaller guys
 - Fly fisherman - cutthroat or rainbow trout
- Tiny guys
 - Activated sludge process controller
 - Bacteria and Archaea
 - Protozoa
 - Metazoa

Microbial Community

- Bacteria and Archaea
 - Up to 99% by mass
 - Feed on soluble and particulate BOD
- Protozoa eat bacteria
 - Amoebas
 - Flagellates
 - Free-swimming ciliates
 - Crawling ciliates
 - Carnivorous free-swimming ciliates
 - Stalked ciliates
- Metazoa eat all the others
 - Rotifers
 - Nematodes (Round Worms)
 - Tardigrada (Water Bears)
 - Annelida (Bristle Worms)
 - Gastrotrichs
 - Ostracods (Seed Shrimps)
 - Cladocera (Water Fleas)
 - Hydrocarina (Water Mites)

Microbial Interactions

- **Mutualism** – dependent on each other
- **Cooperation** – get along, but not dependent
- **Commensalism** – one benefits, other not affected
- **Parasitism** – one benefits, the host is harmed
- **Predation** – attacks and kills prey
- **Amensalism** – adverse effect on the other
 - Bacterial production of antibiotics
- **Competition** – different organisms try to acquire the same resource – important in starving systems such as activated sludge

Bacteria

- Majority of activated sludge biomass
- Grow under “starved” conditions
- High population density
- High surface area to volume ratio
 - High exposure to substrate per organism
 - Access to substrate is key - no mouth!
- Adaptable
 - Can exploit changing conditions
 - Rapidly establish a population
 - “Grow when the growin’ is good” –in its niche
- Density – 1.03-1.056 g/mL – not very heavy

Archaea

- Similar to bacteria
 - Single cell
 - No nucleus
 - Genetically and metabolically different
- Many are extremophiles
 - Extreme temperature, pH, salinity
 - Methanogens (anaerobic digester)
 - Nitrifiers in simultaneous NdN (low DO)
- % of microbial mass????
- Much we do not know!

Example Bacterial/Archaeal Groups

- Aerobes, anaerobes, facultative
- Heterotrophs (organic)/Autotrophs (Inorganic)
- Floc formers/Individual cells
- Nitrifiers
- Denitrifiers
- Phosphorus accumulators – PAOs vs GAOs
- We can design an environment to grow any of these specific groups of critters

Example Approaches to Classifying Bacterial Types

- Origin
 - Source
 - Enteric – 10^8 - 10^9 /g feces
 - Soil
 - Your soil
 - Different plant to plant
 - Environment
 - Growth
 - Niche
- Food
 - Heterotrophic (organic)
 - Autotrophic (inorganic)
- Growth Pattern
 - Floc former
 - Filament
 - Some filaments may grow in non-filamentous form depending on environment
 - e. g., *Sphaerotilus natans*, nocardioforms and *Candidatus Nostocoida limicola*
- Temperature
 - Low – Psychrophilic
 - Medium – Mesophyphilic
 - High - Thermophylic
- Final Electron Acceptor

Final Electron Acceptors

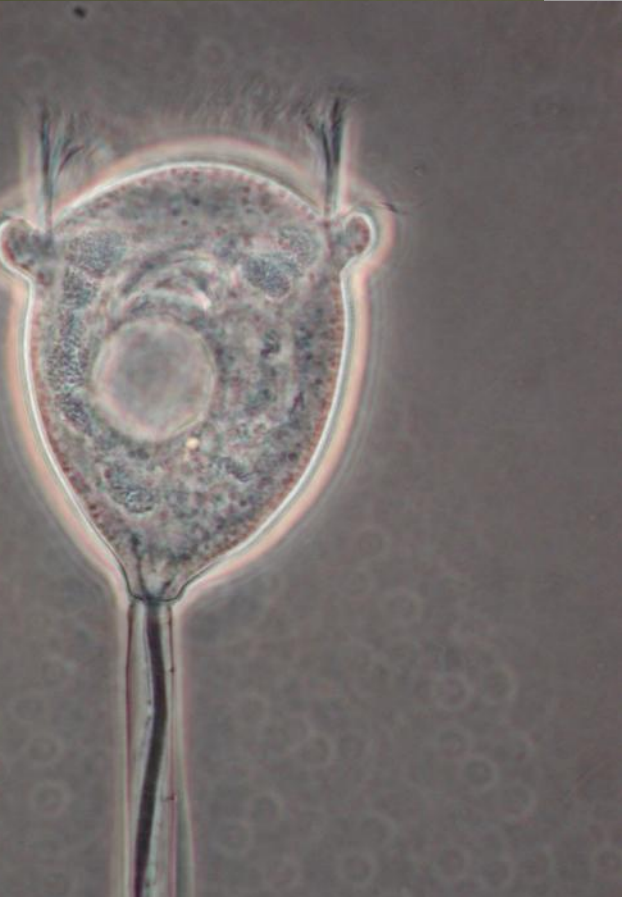
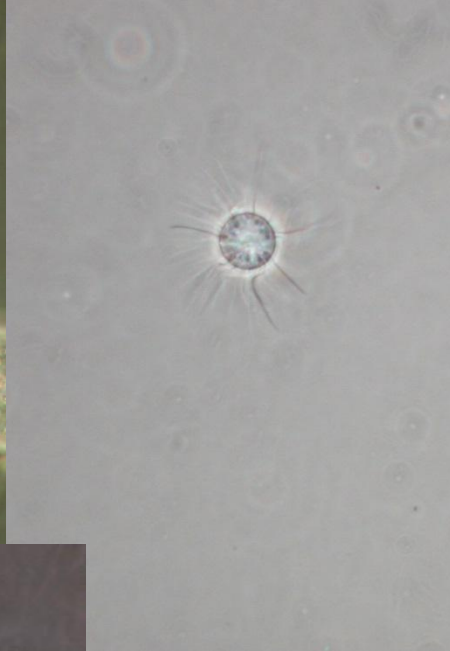
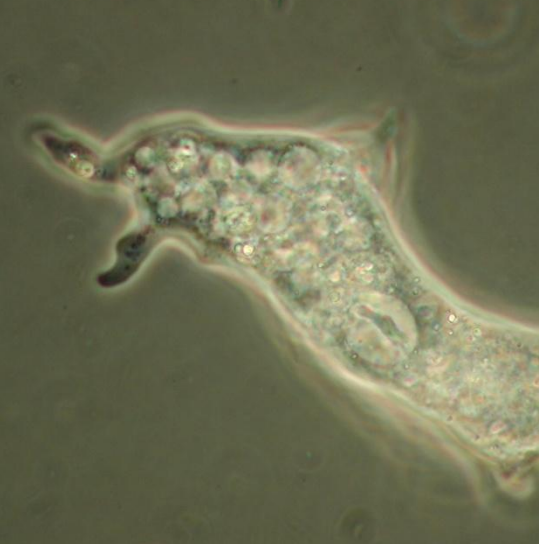
- Obligate aerobe – O_2
 - O_2 required
 - Aerobic respiration
 - Possibly microaerophylic
- Obligate anaerobe – No O_2
 - Fermentation
 - Organic
 - Respiration
 - SO_4^{2-} reduction $\rightarrow H_2S$
 - O_2 may be toxic
 - Aerotolerant
 - O_2 is not toxic
- Facultative Anaerobe
 - Aerobic respiration
 - Preferred metabolism
 - O_2 involved
 - Anaerobic respiration
 - NO_3^- (anoxic)
 - Anaerobic fermentation
 - Organic acid
 - Alcohol

Protozoa and Metazoa

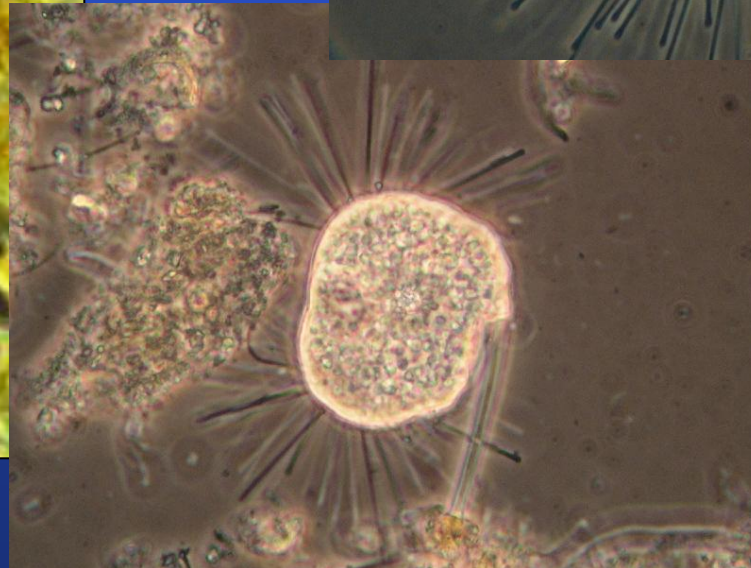
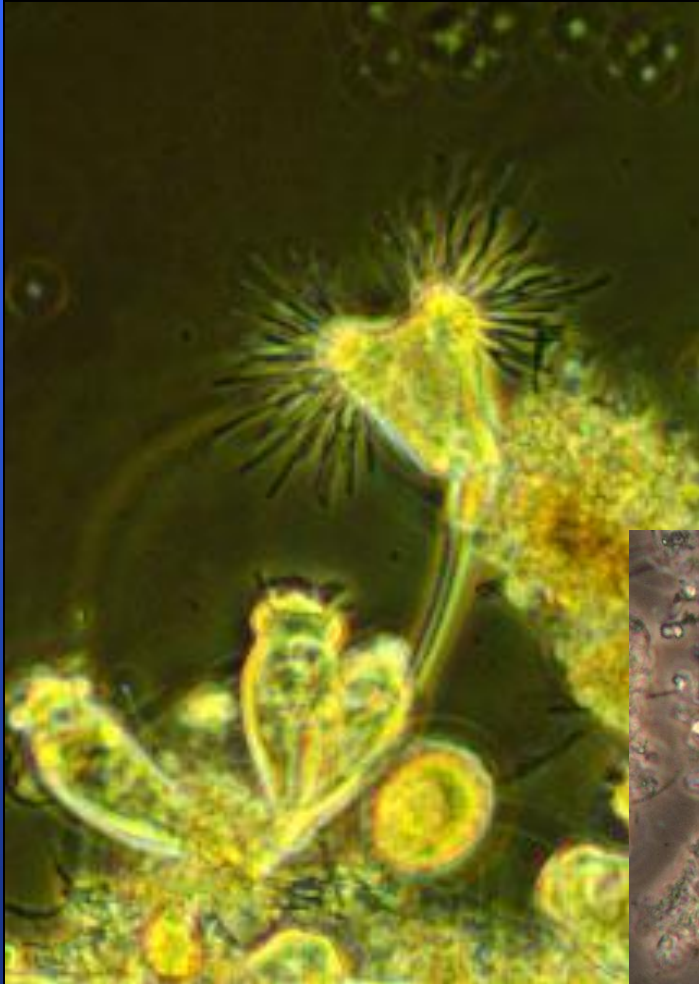
- Present in the Activated Sludge “Neighborhood”
- “Photogenic” - easy to see & recognize
- By mass, very small and insignificant
- Perform some “housecleaning” functions
- Can serve as indicators of the system environment, especially in industrial systems

Protozoa

- Amoebas
- Flagellates
- Bulk-liquid free-swimming ciliates
- Crawling ciliates
- Carnivorous free-swimming ciliates
- Stalked ciliates



A Carnivorous Ciliate - Suctoria



Metazoa

- Multi-celled animals
- Slower growing
- Larger than protozoa
- Do not tolerate ammonia
- Over-oxidized sludge conditions



Metazoa

Too Old

Very low F/M

- Nematodes
- Water Bears
- Bristle Worms
- Gastrotrichs
- Ostracods
(Seed Shrimps)
- (Water Fleas)
- Hydrocarina
(Water Mites)

• Rotifers



Niche & Outcompete

- Niche: Any unique environmental combination in which an organism can grow:
 - Temperature
 - pH
 - Food source (and concentration)
 - Available electron acceptor
 - Other
- *Someone is ALWAYS at home, ready to grow*
- Microorganisms (bacteria) are incredibly adept at exploiting niches – *population, size, growth rate*
- → We have some CONTROL to influence the “niches” created in the activated sludge environment

SUMMARY

- Organisms relate to each other – mutualism and competition usually the most important
- Bacteria/Archaea do most of the BOD removal
 - Utilize food (substrate) containing carbon
 - Carbon transferred to cells or CO₂
- Bacterial form, shape, and condition affect settleability
- Few can be identified microscopically
 - Filaments
- Many other organisms at home in an Activated Sludge environment
 - Protozoa
 - Metazoa
- How do we control their growth?

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