

A Laboratory Program  
for Wastewater  
Microbiology



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# Why Microbiology?

- It is important to develop and maintain a process control program that includes wastewater microbiology.
- Conducting simple analyses, routinely and consistently is key to understanding the microbiological workings of the wastewater treatment plant.

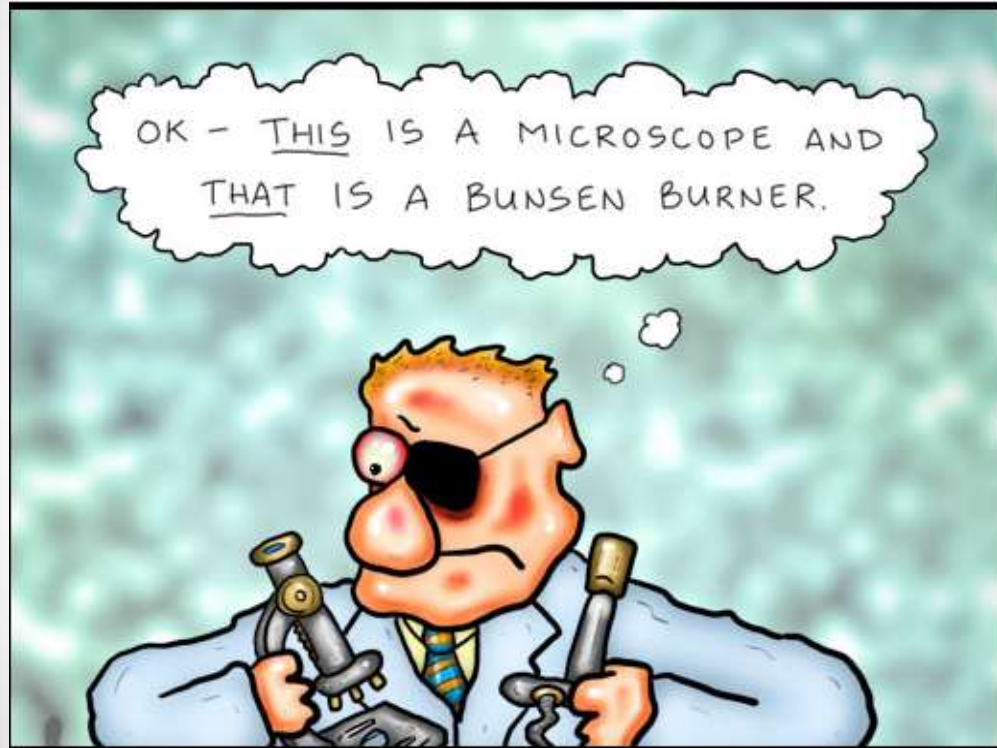
# The Problem

- Many facilities do not have dedicated laboratory staff
- The main focus is on permit related compliance monitoring



# Typical Process Monitoring

- BOD
- TSS
- MLSS
- MLVSS



# Typical Process Monitoring

- 30-Minute  
Settling Test



# Typical Process Monitoring

- Sludge Volume Index (SVI)
- Typically used to monitor the settling characteristics
- The volume (in milliliters) that is occupied by one gram of mixed liquor after 30 minutes of settling.



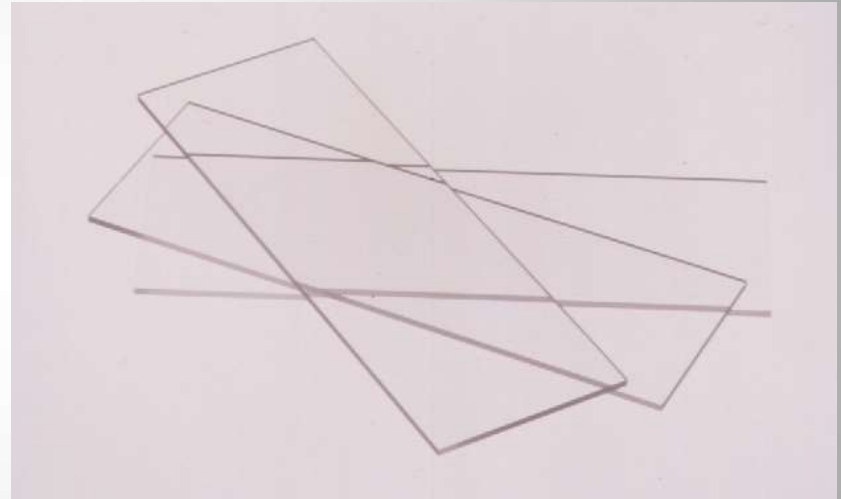
# A Simple Laboratory Program

- Floc Color Index
- Zooglear Mass Count
- Total Shelled Protozoa-Metazoa
- Total Filament Count
- SVI
- MLVSS



# A Simple Laboratory Program

- Wet Mount
  - Floc Color Index
  - Total Shelled Protozoa
  - Zooglear Mass Count
- Stained Slide
  - Filament count
  - Zooglear Mass Index

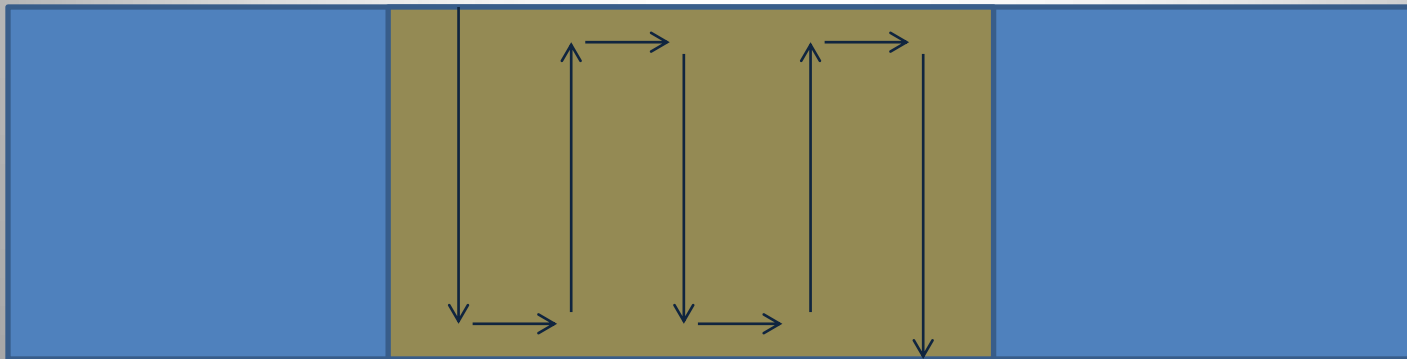
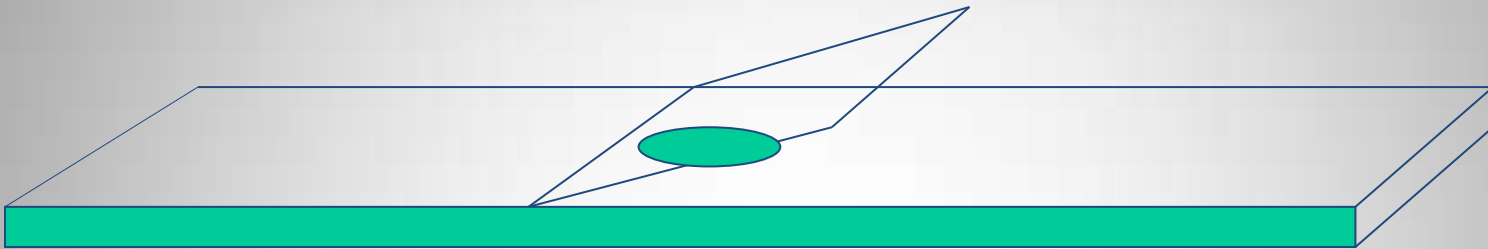


# A Simple Laboratory Program

- Always use a well mixed, representative sample of mixed liquor
- Always use the same volume or number of drops on the slide.
- Always collect the sample from the same location
- Use the same sample for all the tests

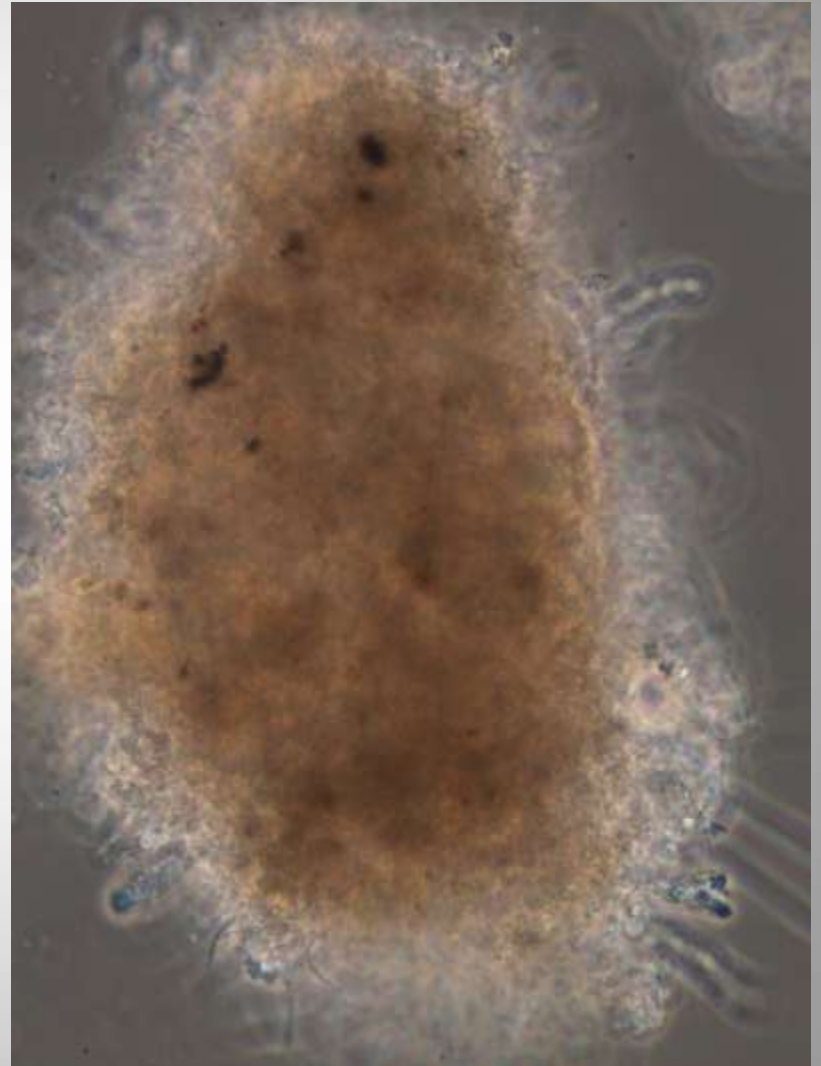


# Wet Mount & Scan

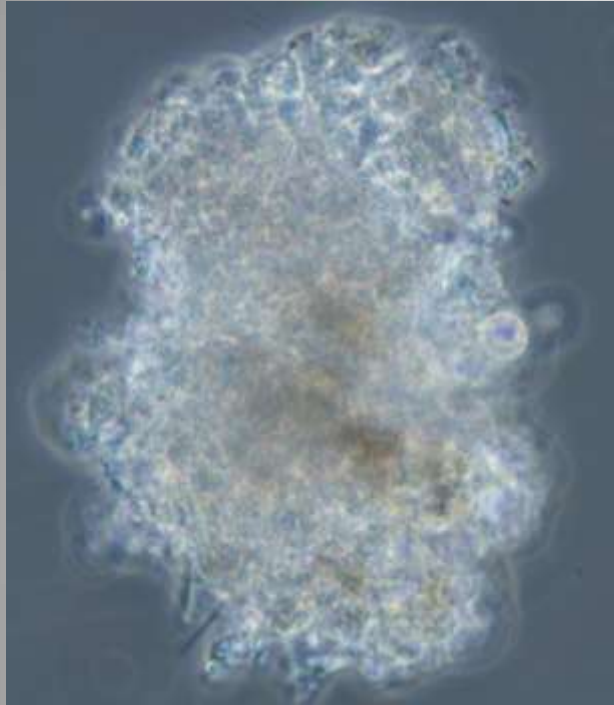


# Floc Color Index

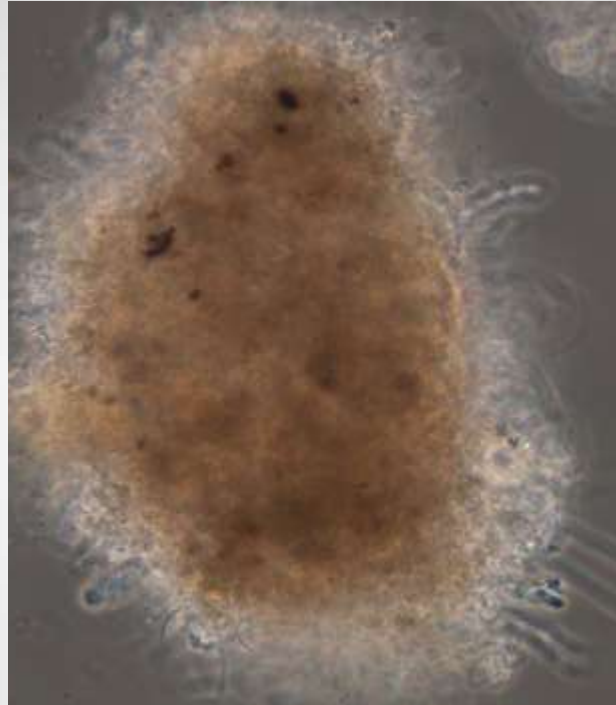
- Under normal conditions floc should appear brown when observed using phase contrast.



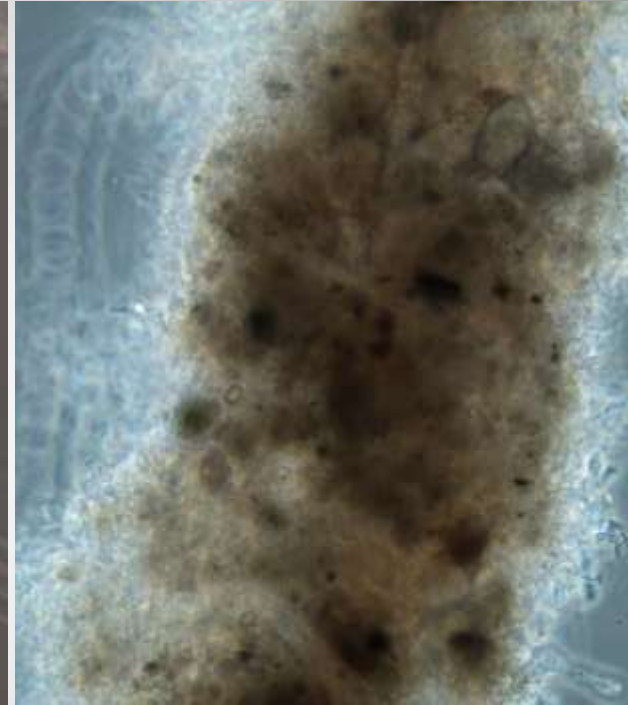
# Floc Color Index



-5



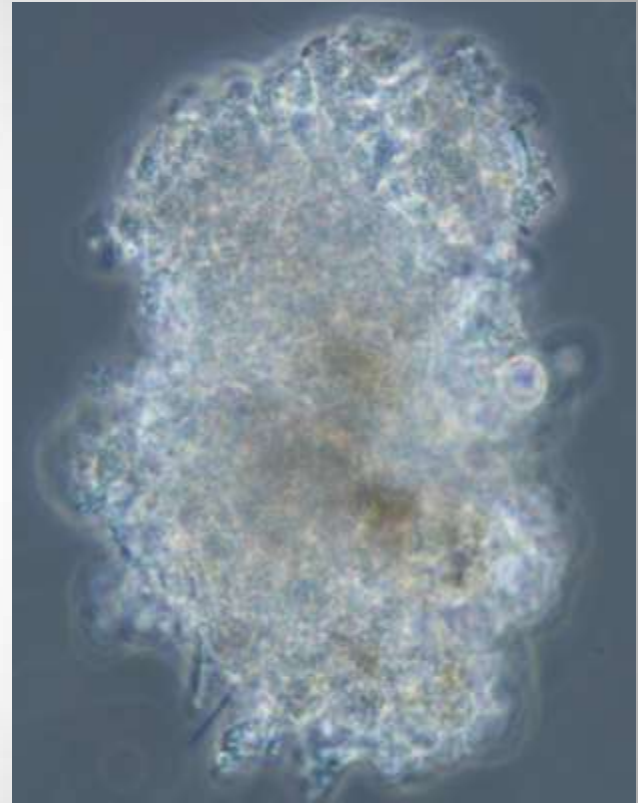
0



+5

# Floc Color Index

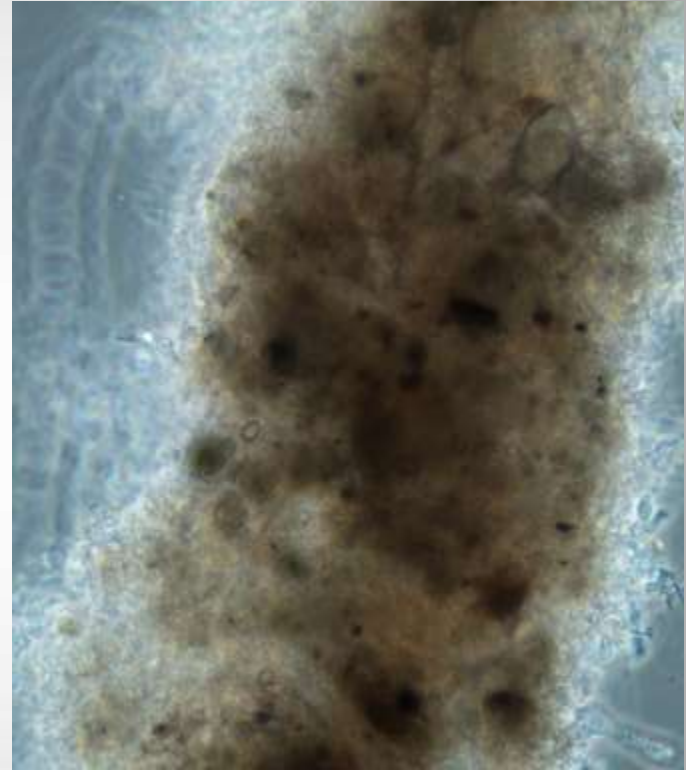
- Low food
- Low nutrients
- Under low food/low nutrient conditions bacteria secrete excess lipopolysaccharide
- The more starved they are, the more they secrete



-5

# Floc Color Index

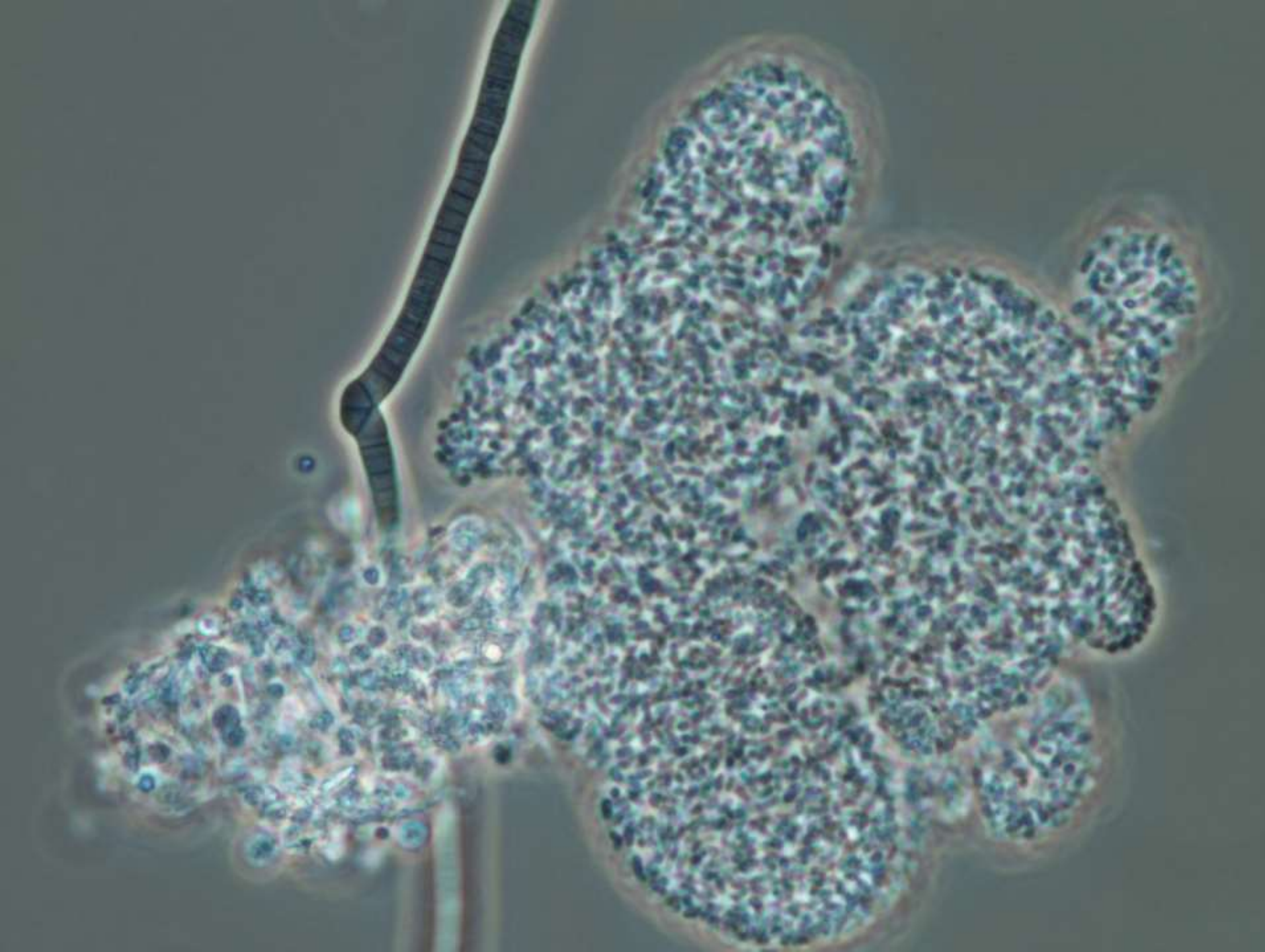
- Old Sludge
- Septic Sludge
- Older sludge is darker in color.
- When oxygen is lacking sludge becomes septic and turns dark brown to black in color.

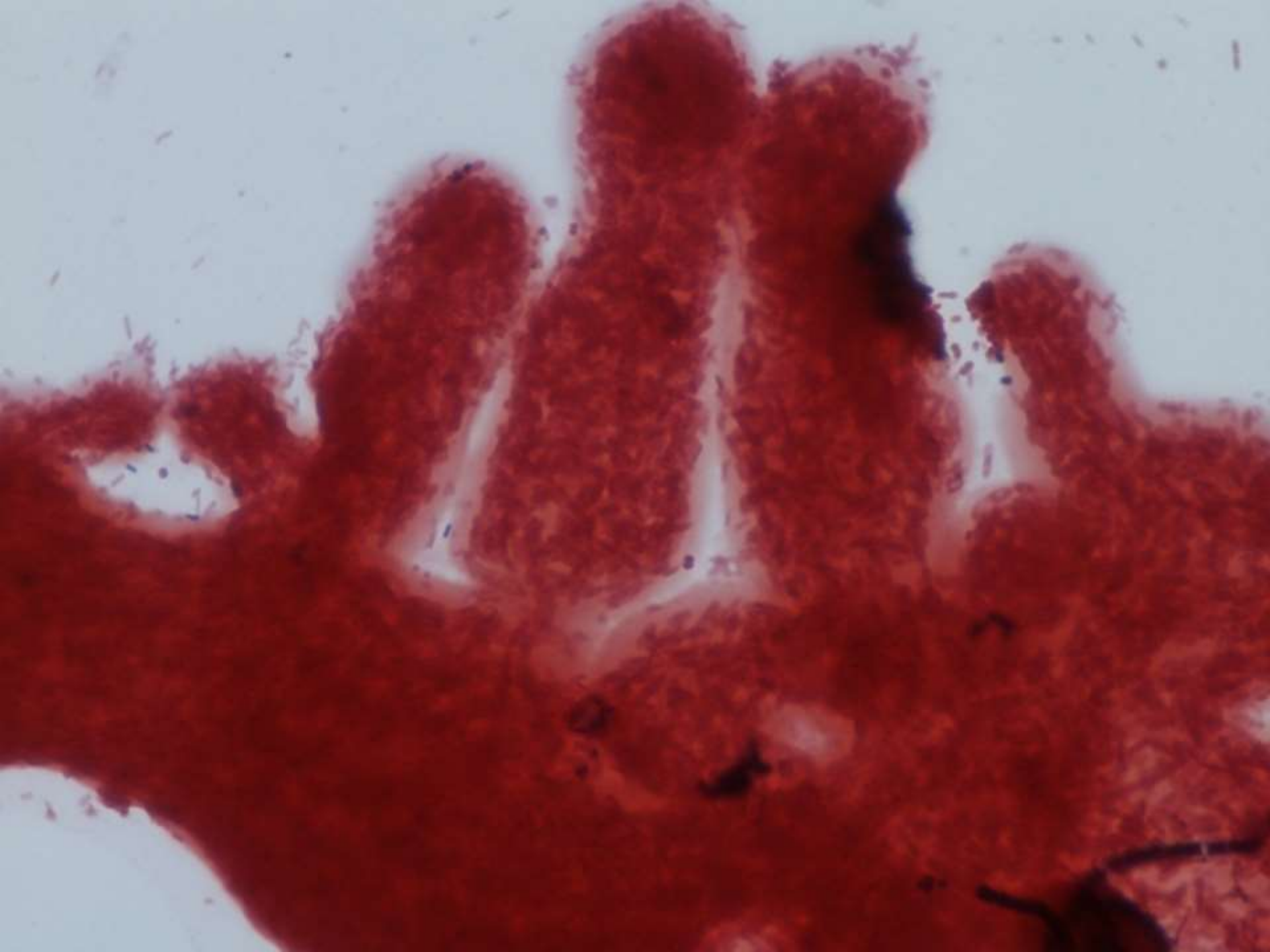


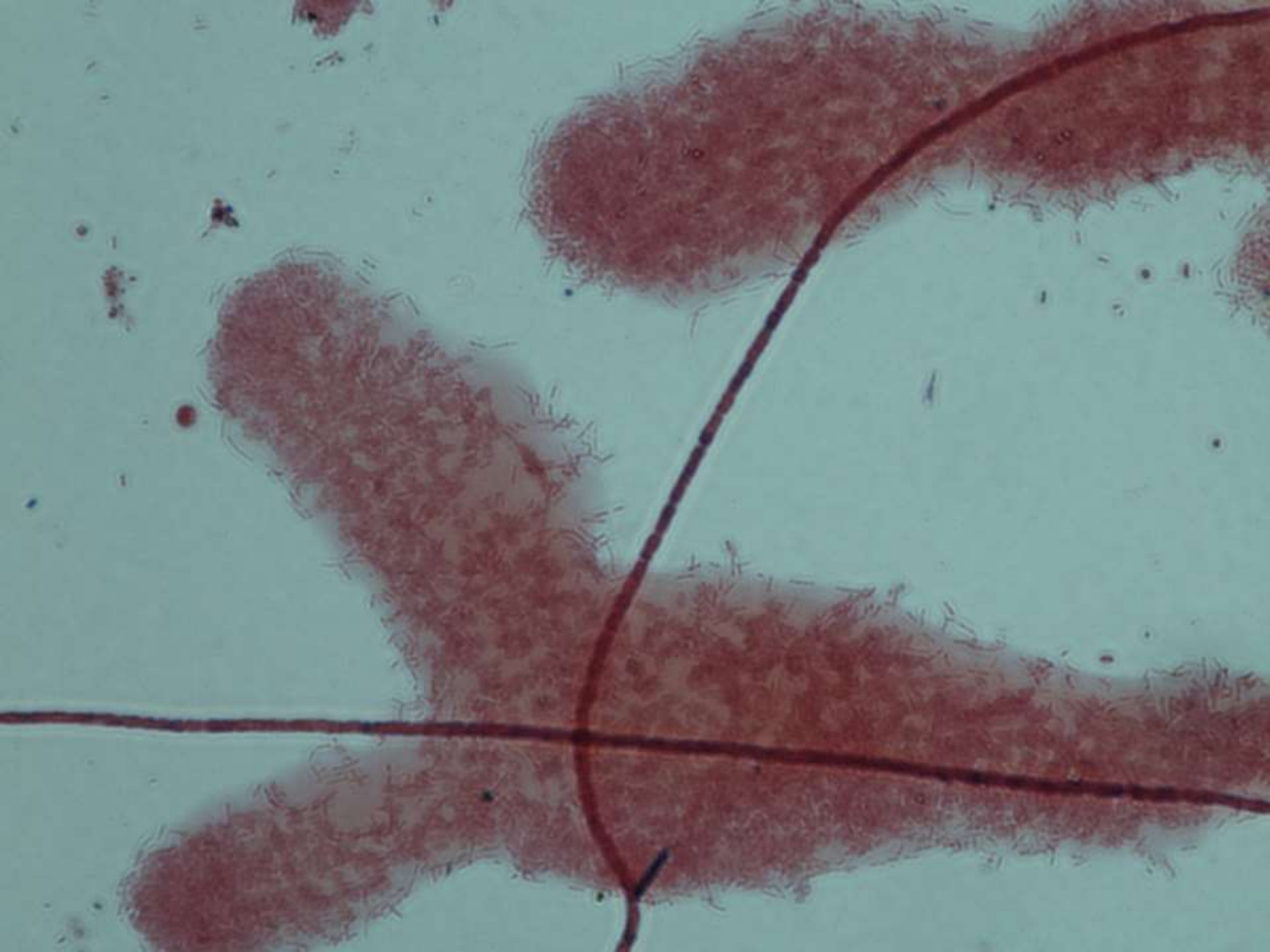
+5

# Zoogleal Mass Index

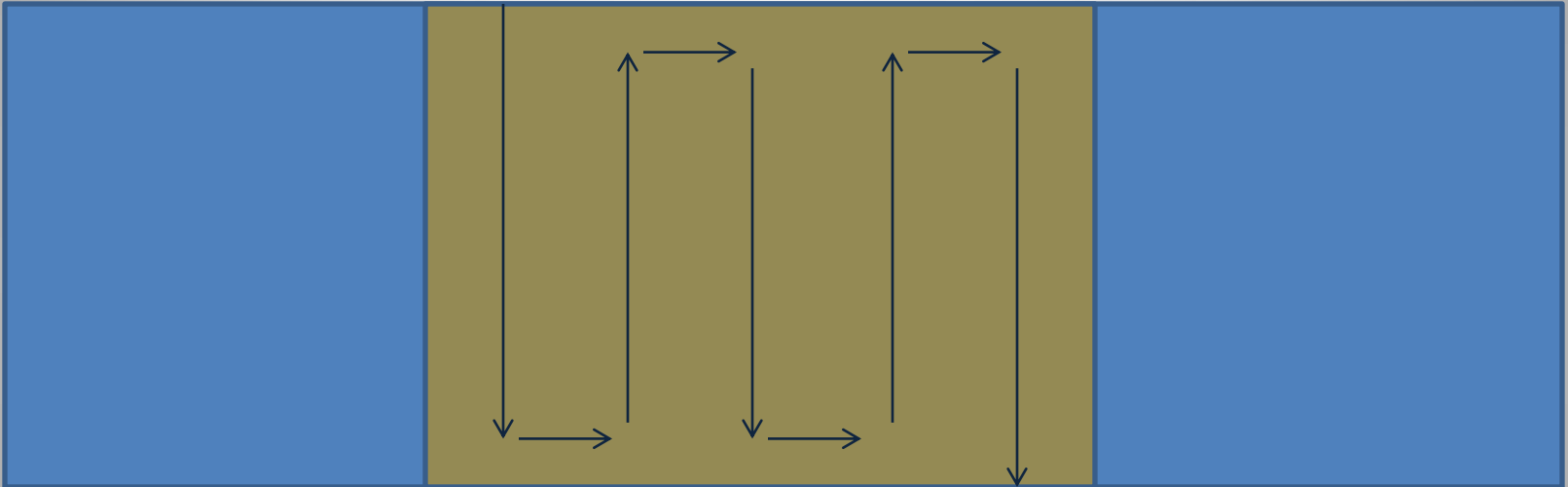
- There are basically two types of bacteria in the treatment system.
  - Those that form floc and those that do not
- Floc-formers generally react to negative situations by producing excess amounts of lipopolysaccharide.
- Non floc-formers generally form zoogleal masses in response to negative conditions.







# Zoogleal Mass Index



# Zoogleal Mass Index

$$\frac{\text{Total Number of Masses Counted} \times 5}{\text{MLVSS}} \times 1000$$

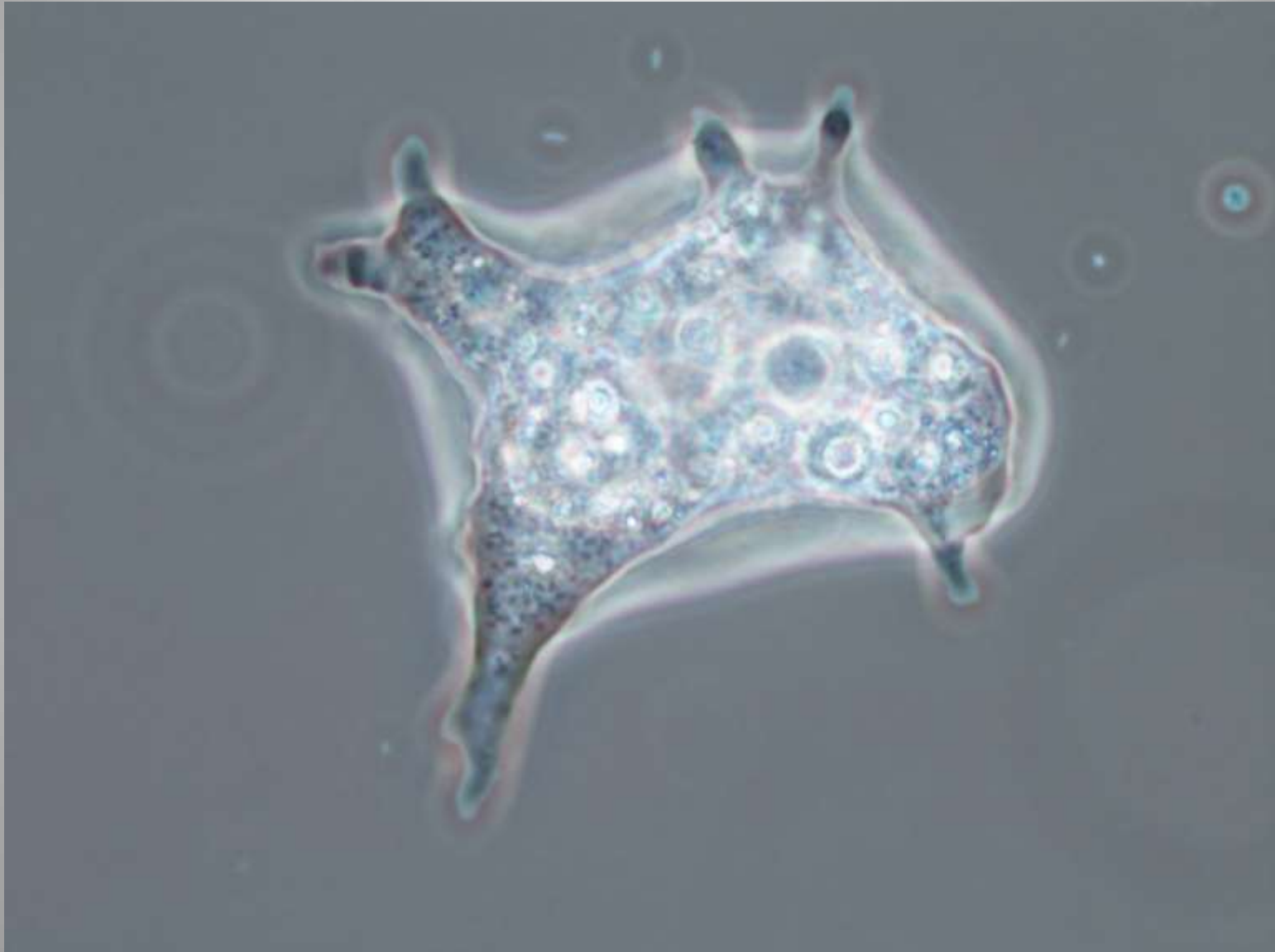
Zoogleal Mass Index/mgVSS

# Shelled Protozoa & Metazoa

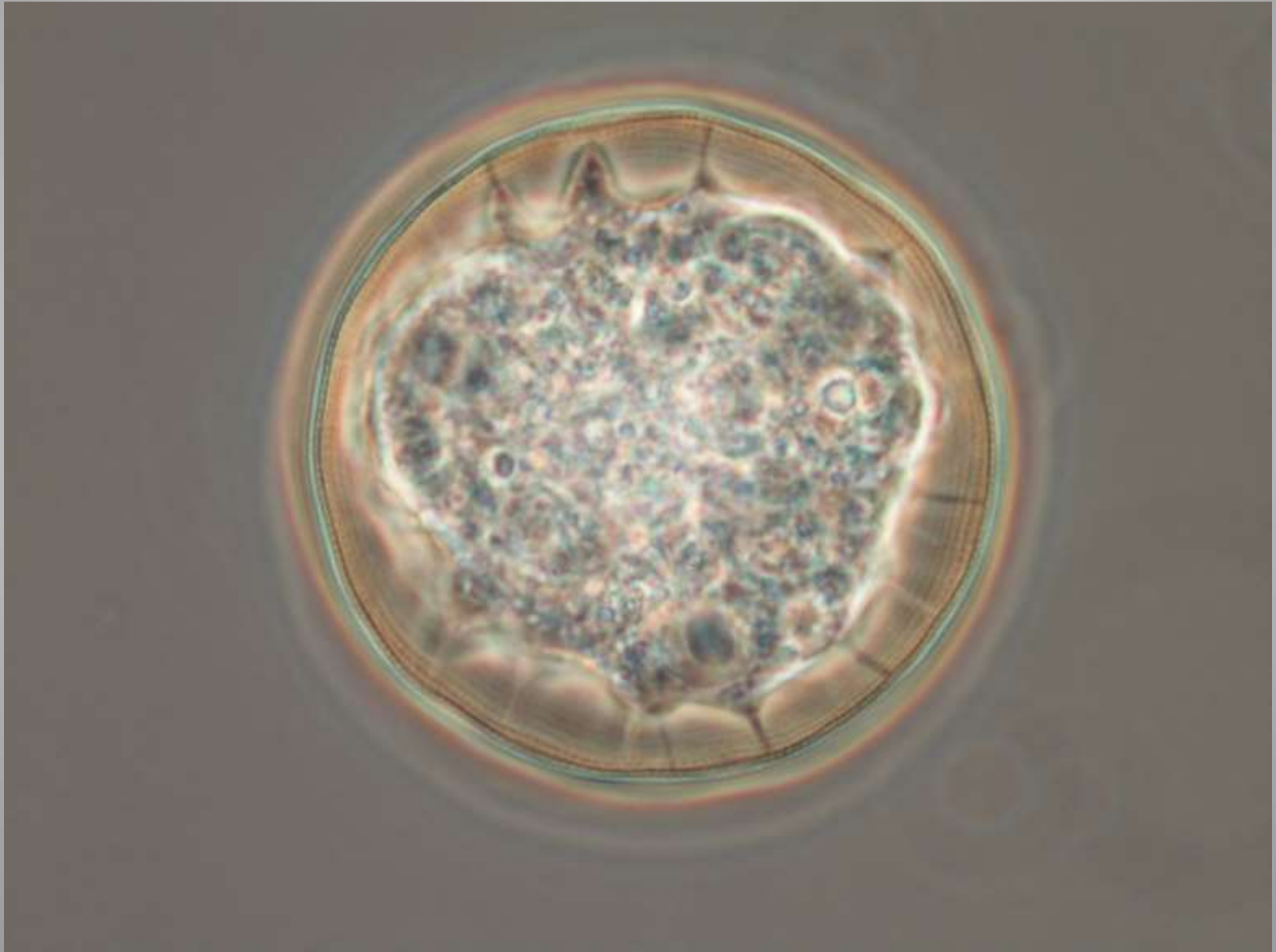
- When conditions are unfavorable protozoa and metazoa that form shells will dominate in the system.
- Conditions such as low levels of toxicity, changes in pH etc.



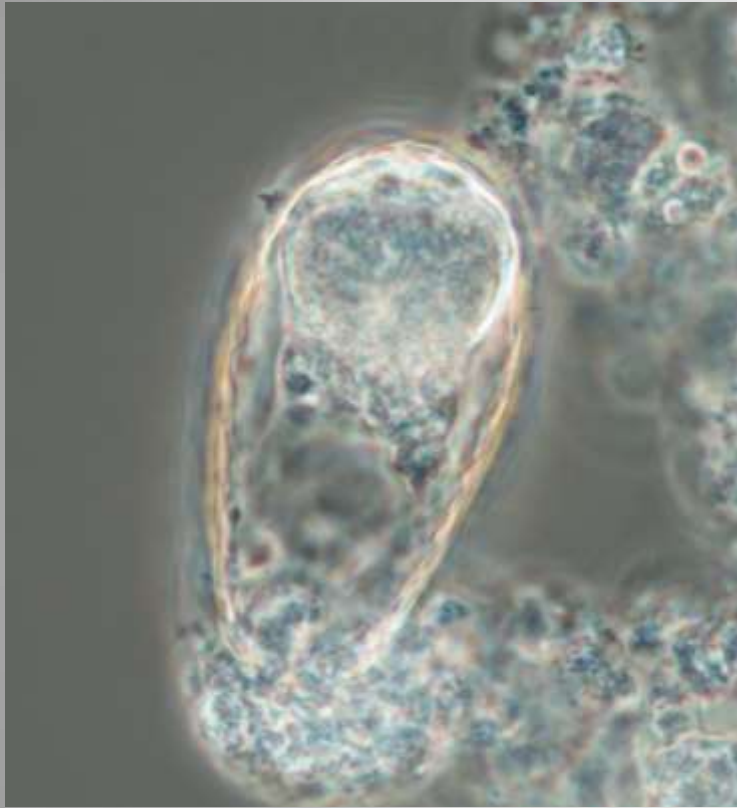
# Shelled Protozoa & Metazoa



# Shelled Protozoa & Metazoa



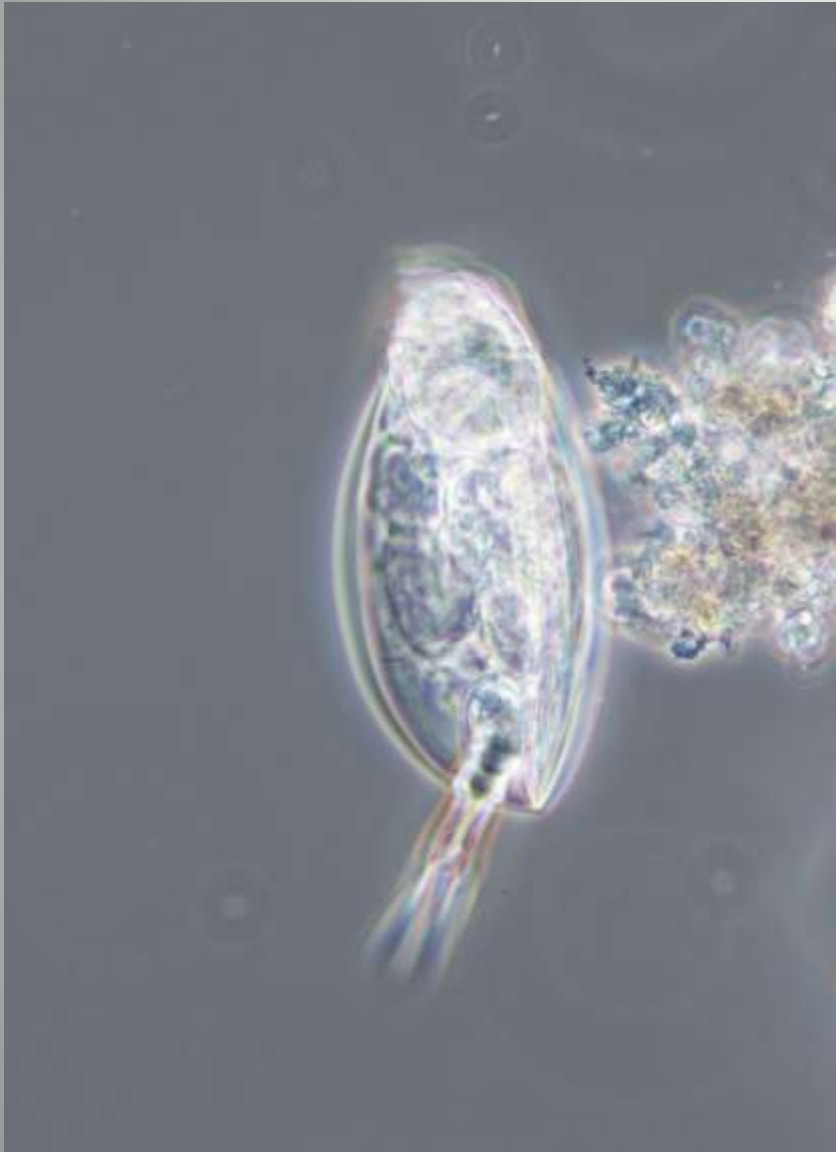
# Shelled Protozoa & Metazoa



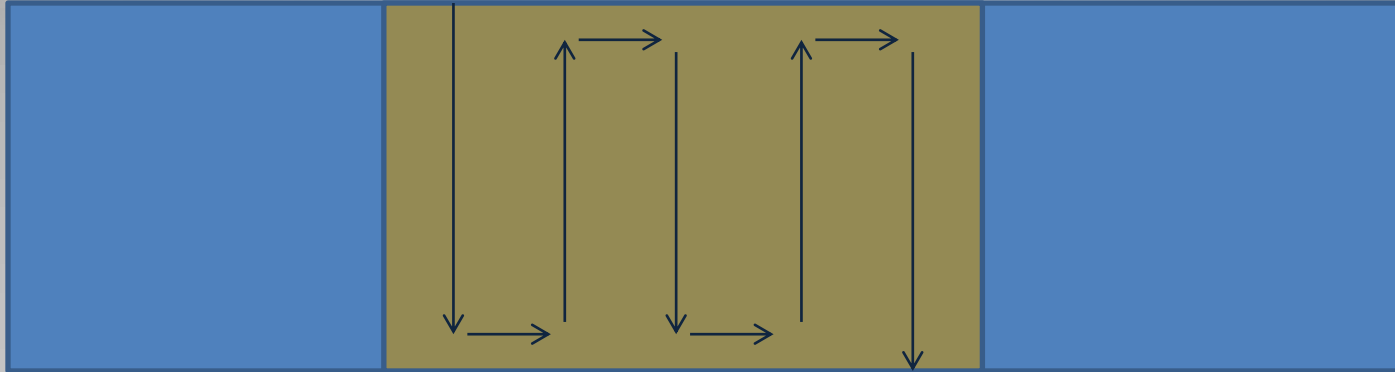
# Shelled Protozoa & Metazoa



# Shelled Protozoa & Metazoa



# Shelled Protozoa & Metazoa



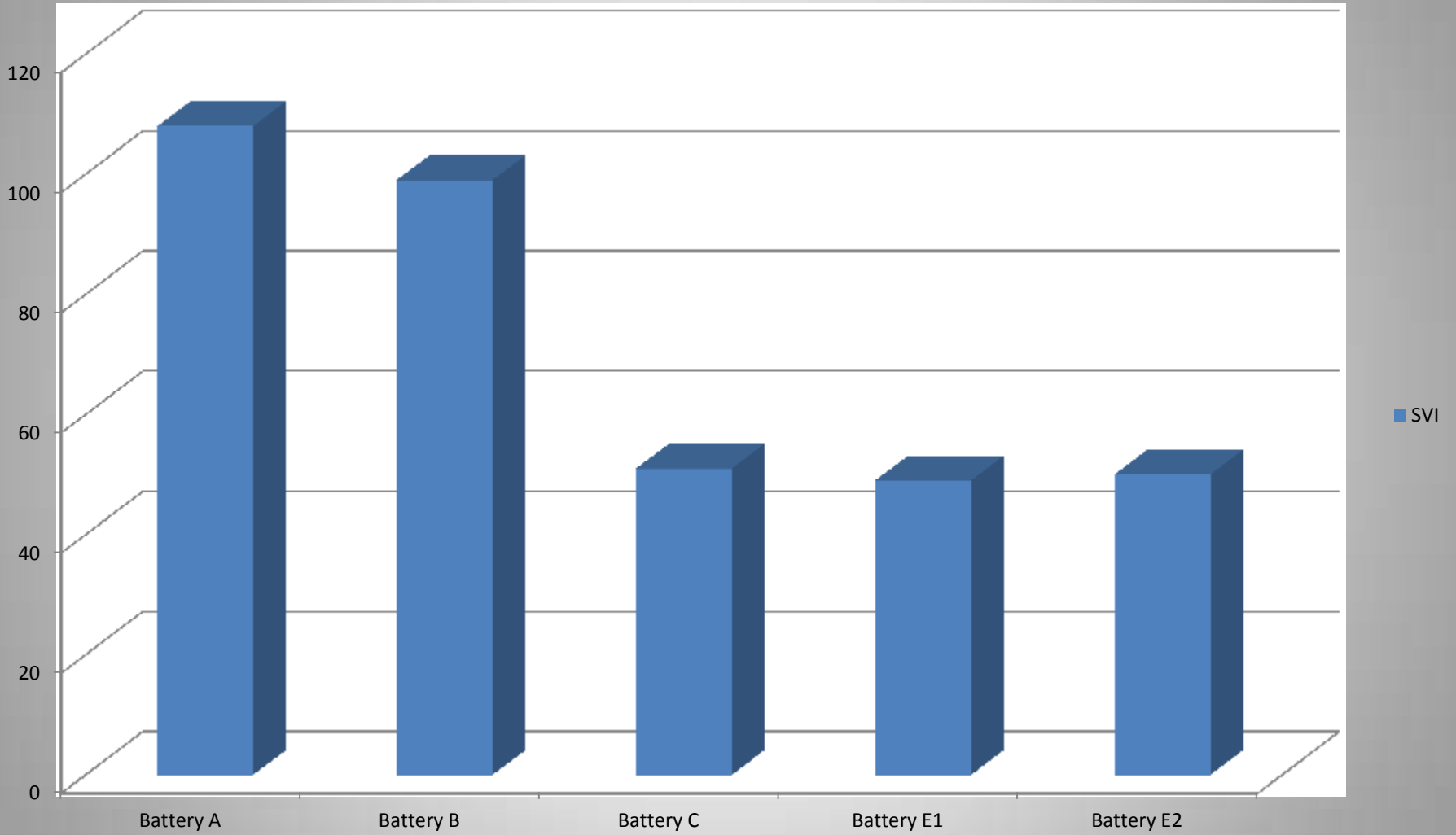
- Count the protozoa and metazoa (un-shelled)
- Count the shelled protozoa and metazoa
- $\text{Shelled} / \text{Shelled} + \text{Un-shelled} = \% \text{ Shelled}$ 
  - <25%
- Total shelled protozoa and metazoa

# Plant A

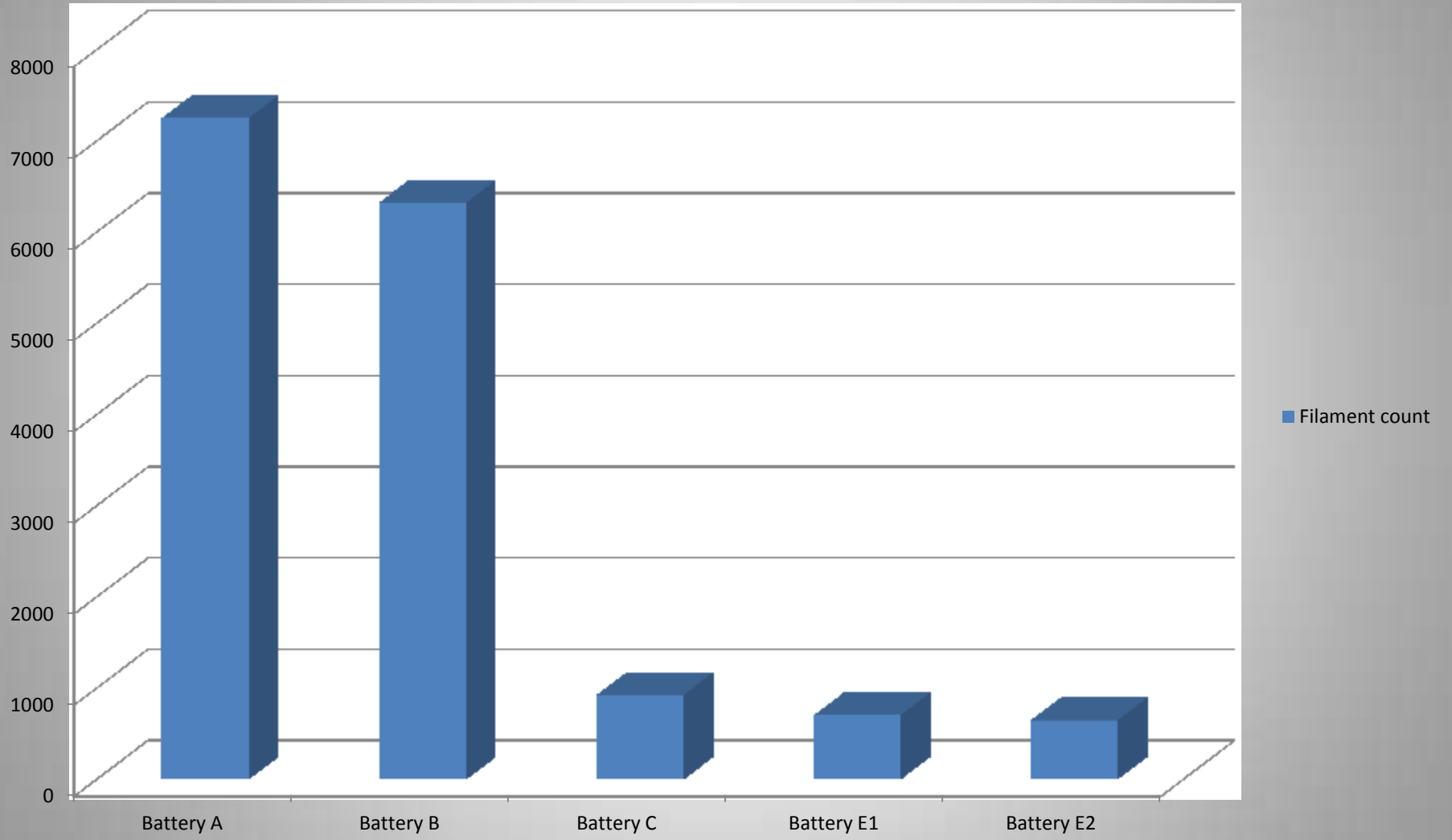
- Five Batteries
- 3 Distinct configurations
  - Battery A and B
  - Battery C
  - Battery E-1 and E-2



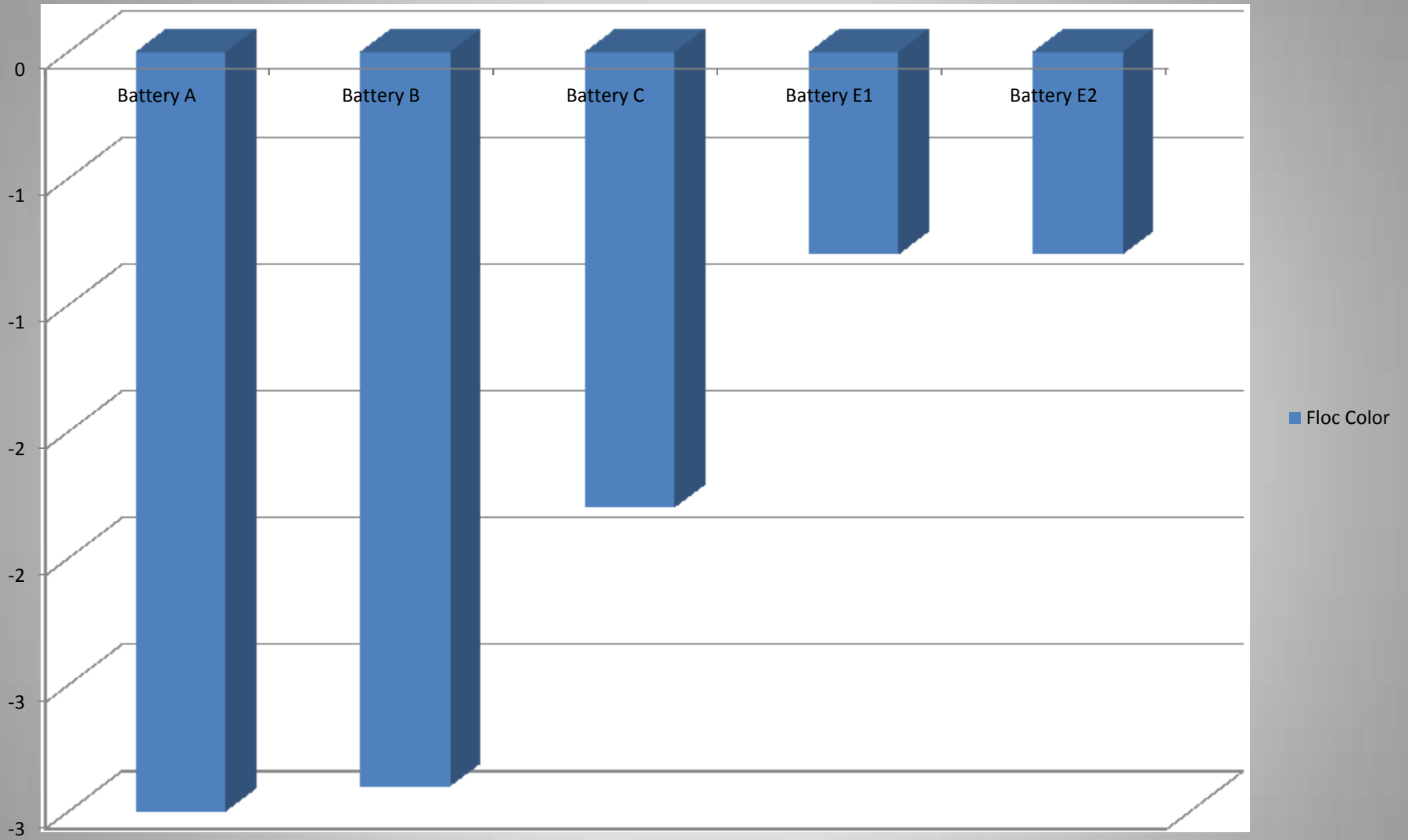
# SVI



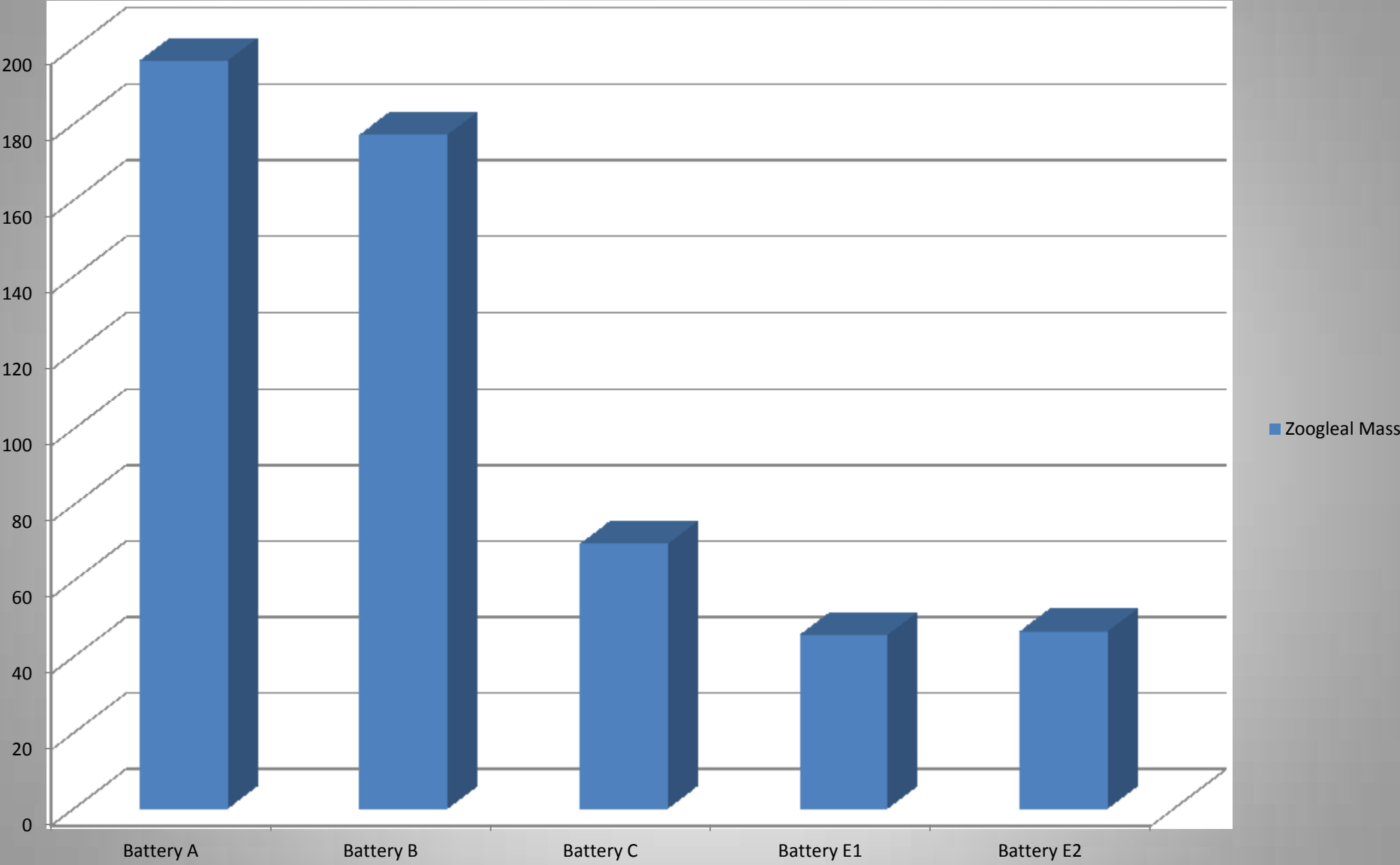
# Filament count



# Floc Color

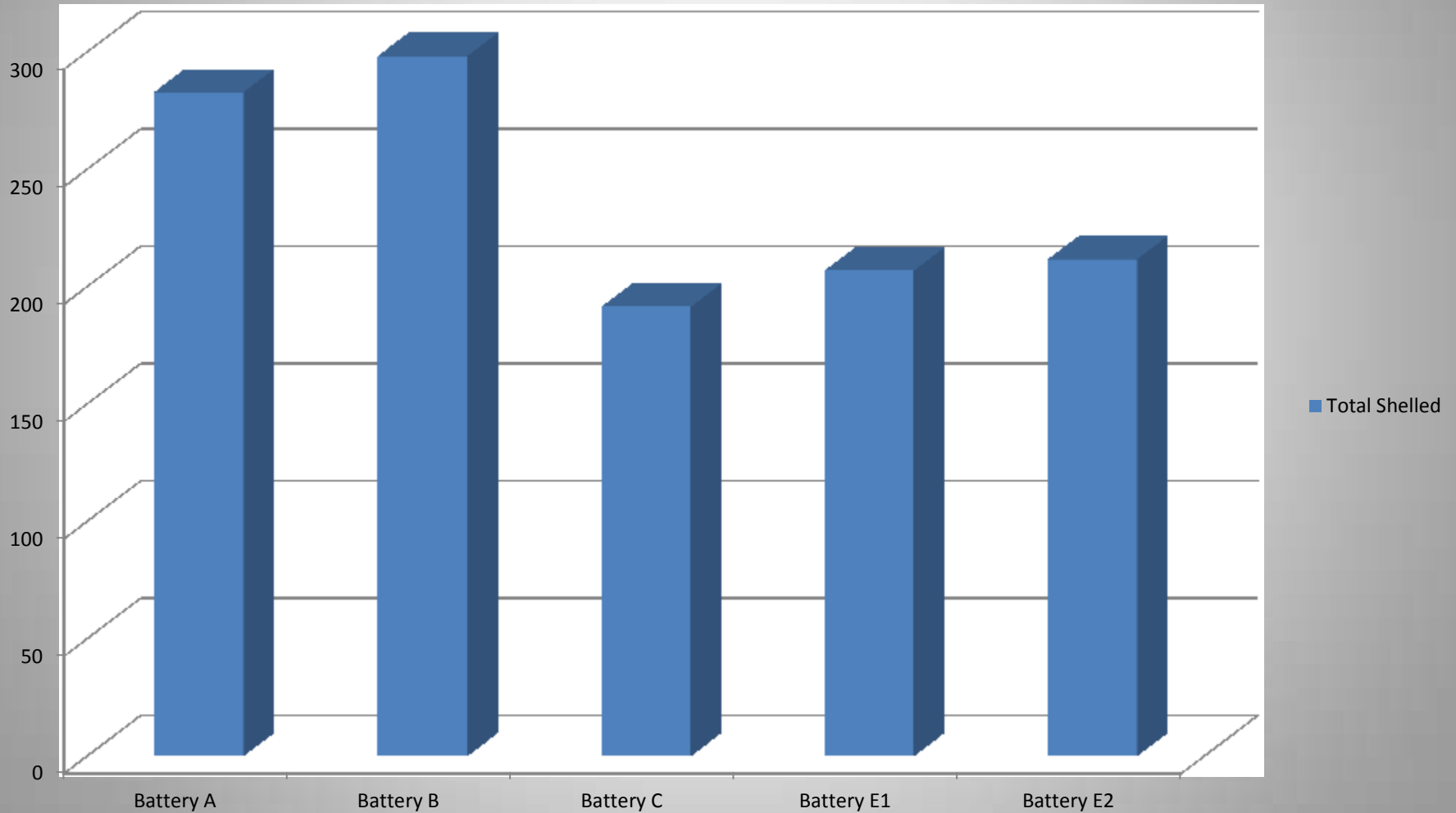


# Zoogleal Mass

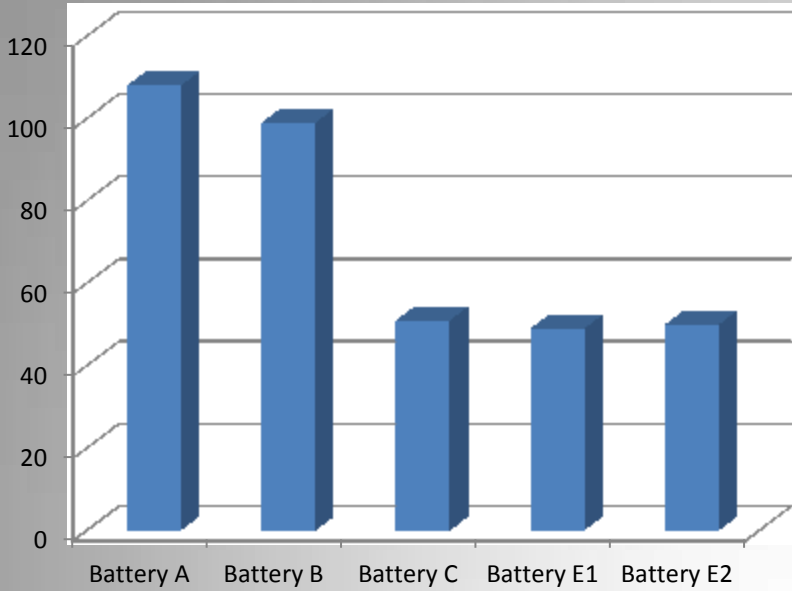


# Shelled Protozoa & Metazoa

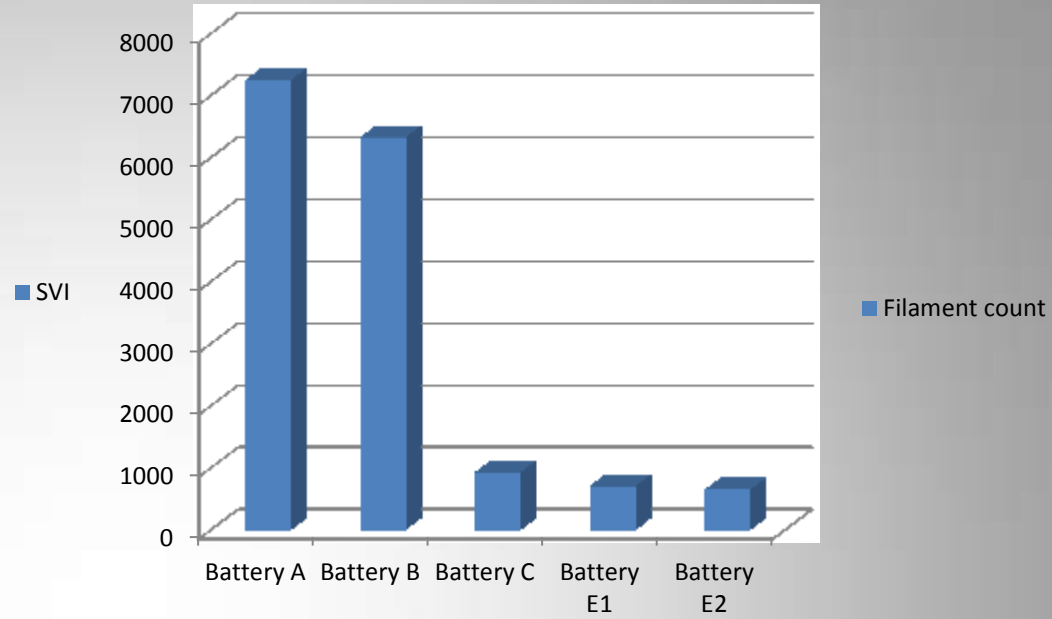
Total Shelled



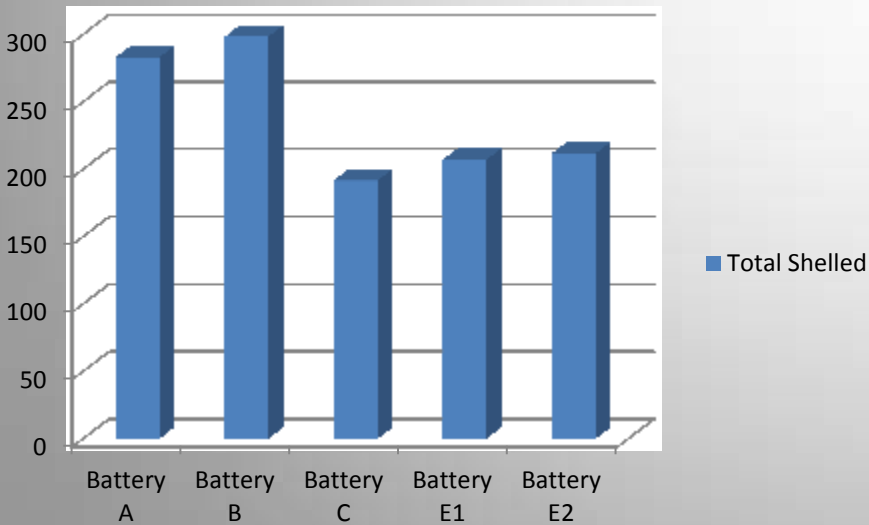
### SVI



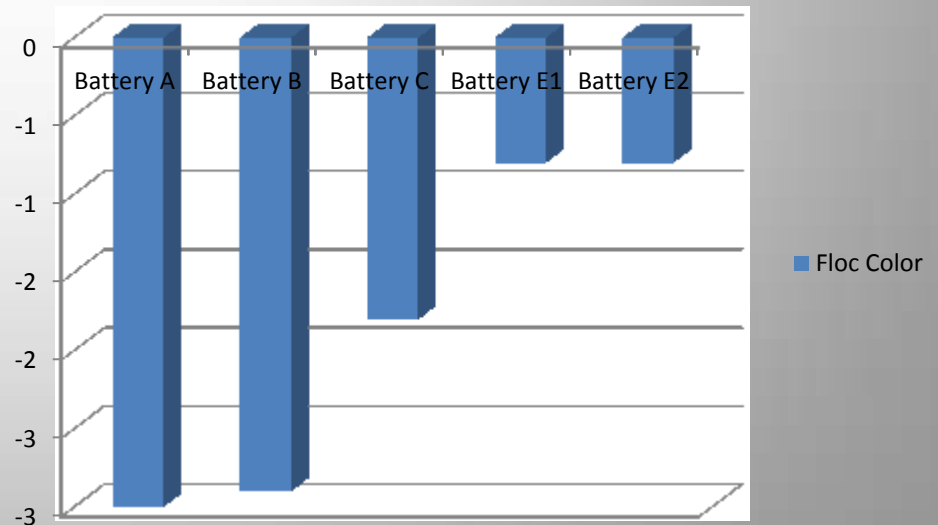
### Filament count



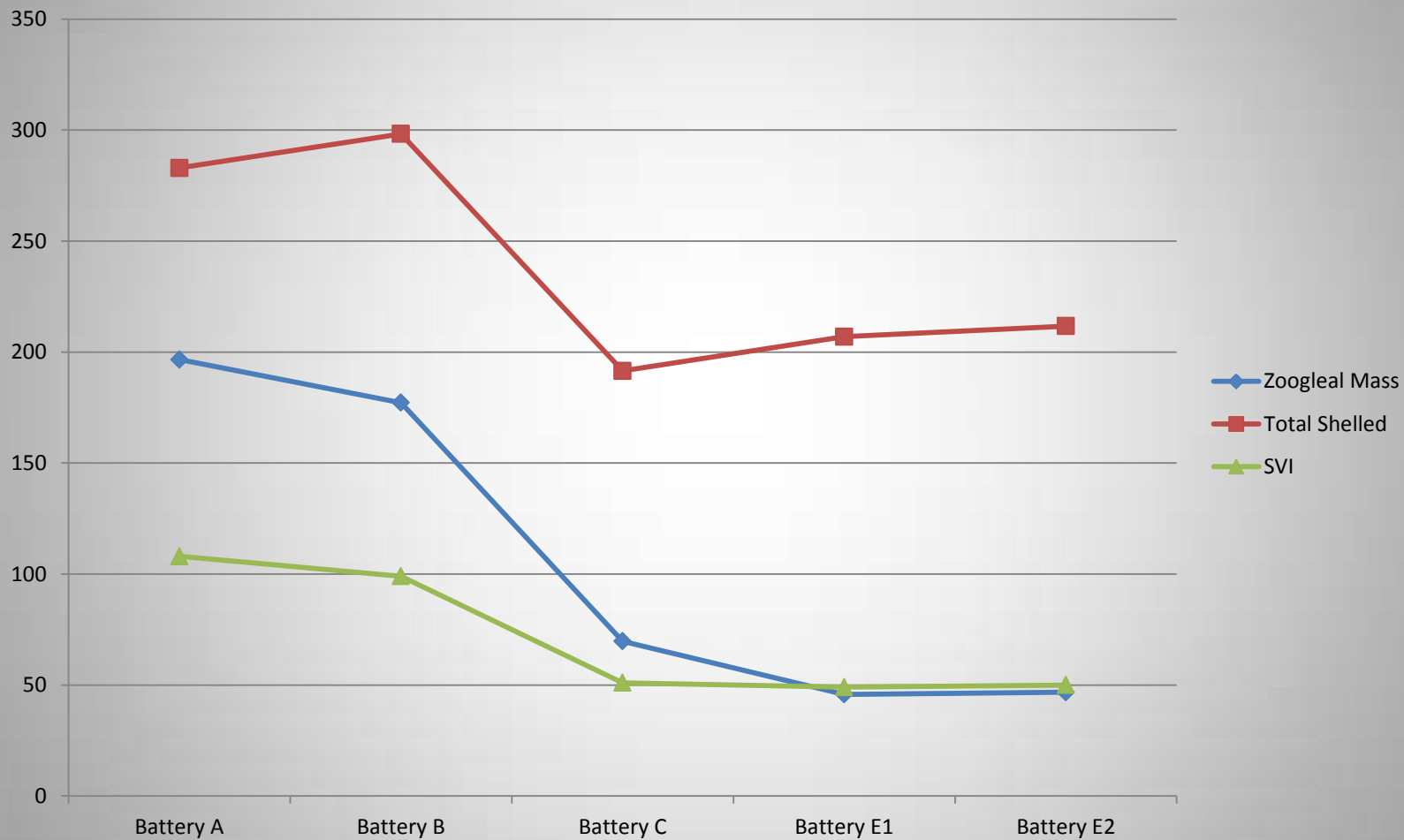
### Total Shelled



### Floc Color



# Plant A



# Why Microbiology?

- Looking at only one parameter does not give you the whole picture.

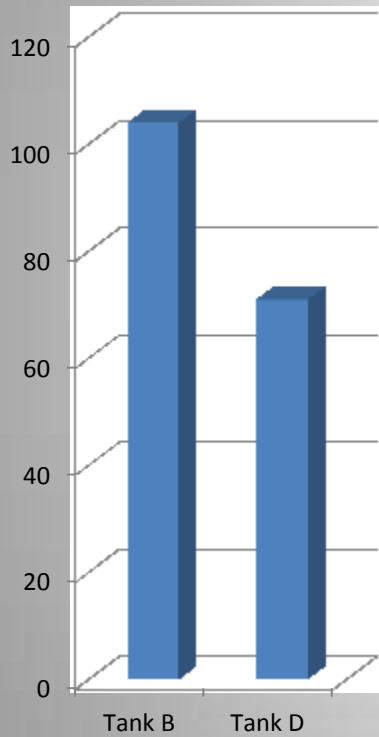


# Why Microbiology?

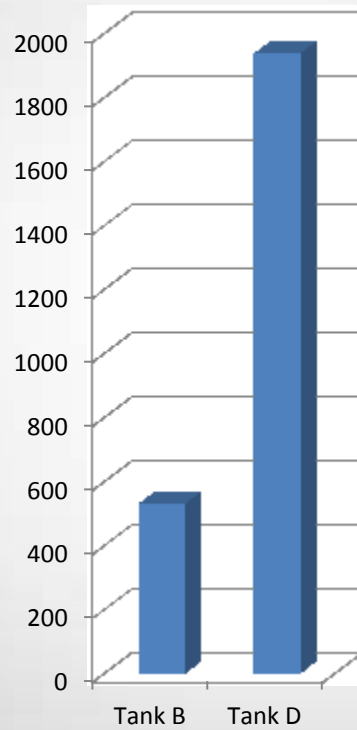
- The SVI or 30-minute settling tests tell you there is a problem but may not necessarily tell you what the problem is.
  - Filamentous Bacteria
  - Excess Zooglea
  - Slime Bulking

# Plant B

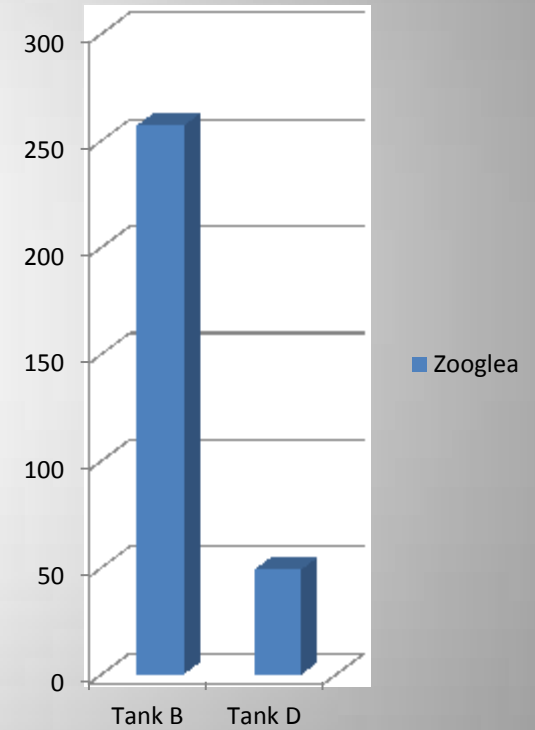
## SVI



## Filament

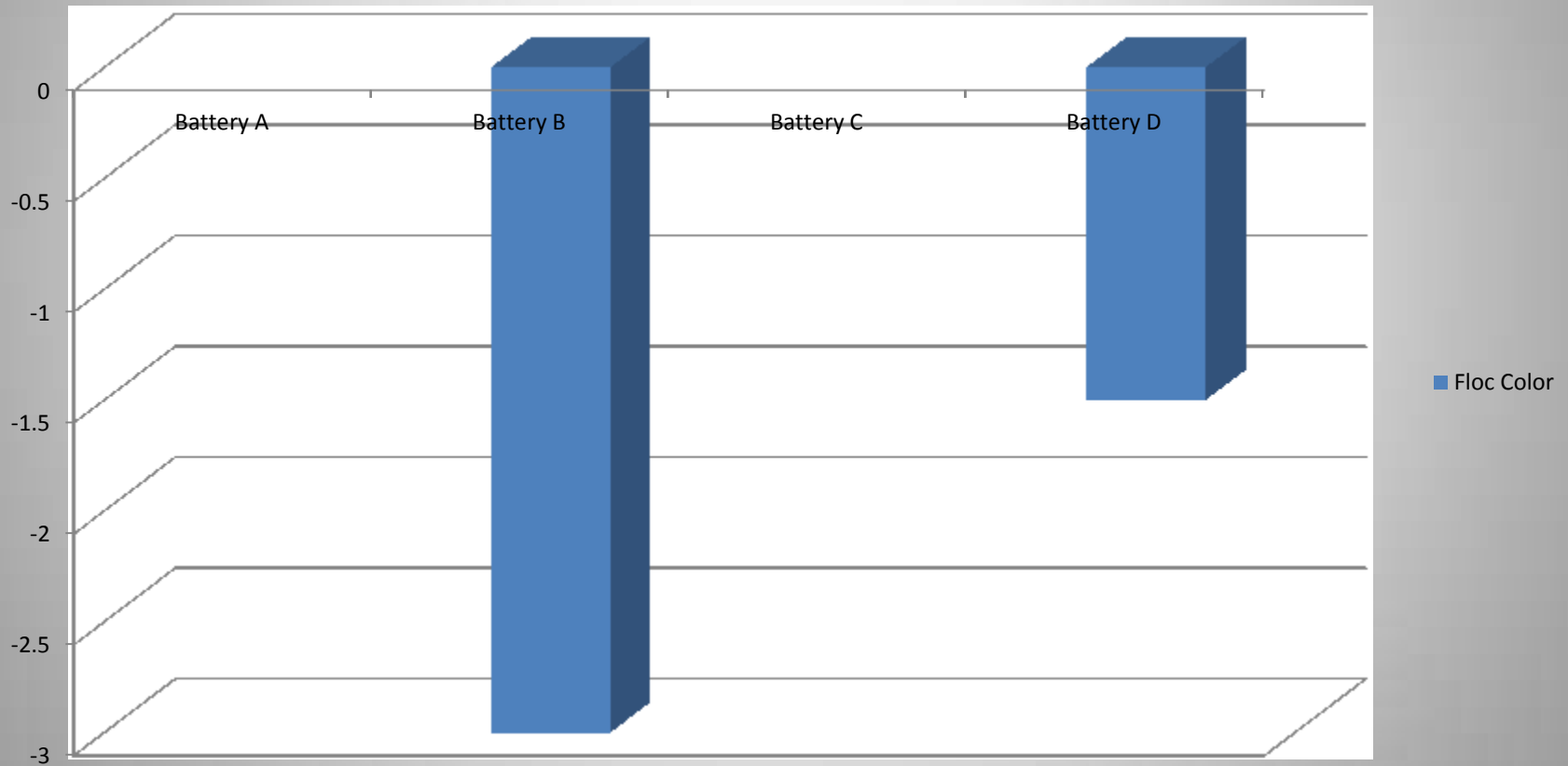


## Zooglea



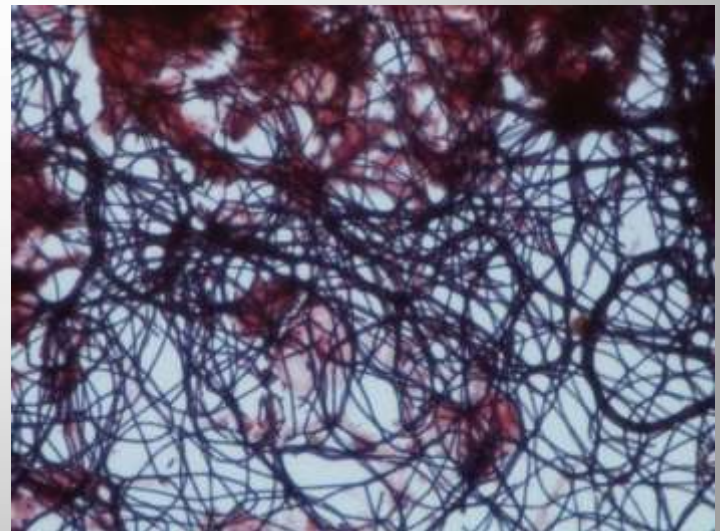
# Plant B – Floc Color

Floc Color



# Filamentous Bacteria Identification

- For the untrained eye, those that are impatient, or who do not have time, identifying filamentous bacteria can be tedious and time consuming.



# Filamentous Bacteria Identification

- Instead of going through the tedious process of determining cell shape, size, length, width, sheaths, branching, attached growth etc. Lump them together based on the conditions best suited for their growth.

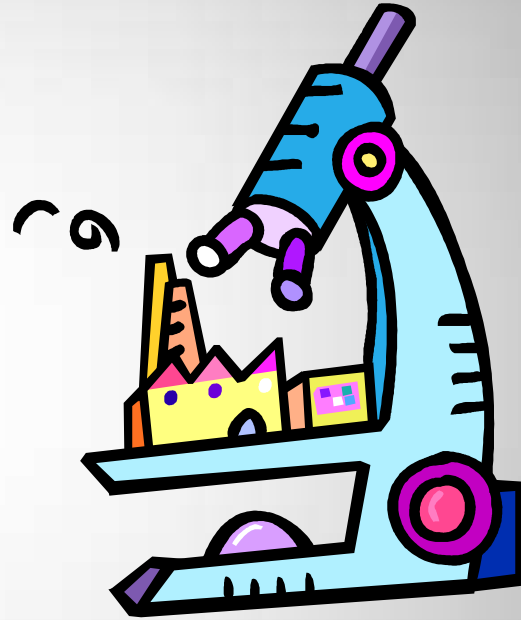


# Filamentous Bacteria Identification

- Make a smear and Gram stain the slide.
- Look at the slide using the oil immersion lens and look at the *Filament Identification Guide* and find a match.
- If it looks like the picture, then that is probably what it is.
- If the filament is in the low DO category then that condition most likely contributed to its growth in the system.

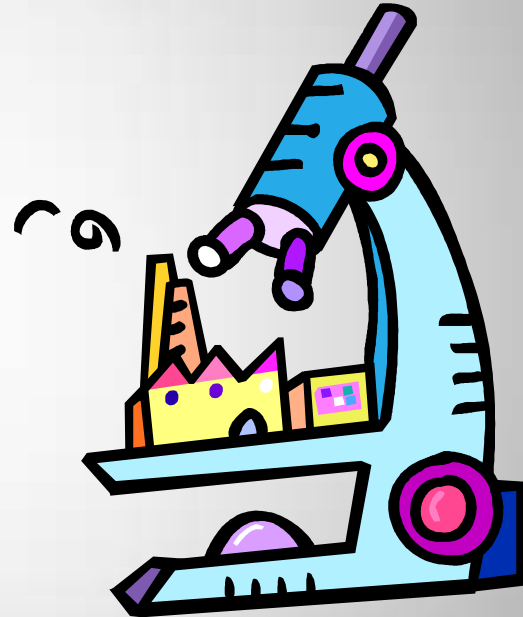
# A Simple Laboratory Program

- Developing the habit of looking at the microorganisms on a regular basis can be hard, particularly given time restraints and dwindling resources.
- Some observations is better than no observations at all.



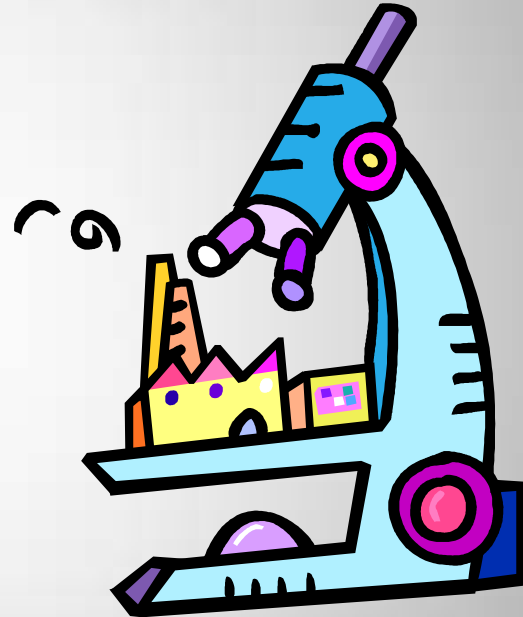
# A Simple Laboratory Program

- If you only have time to look at the floc, then look at the floc; but be consistent.
- If you choose to do protozoan counts, be consistent.



# A Simple Laboratory Program

- If you only have time to look at the floc, then look at the floc; but be consistent.
- If you choose to do protozoan counts, be consistent.



# A Simple Laboratory Program

- Do it weekly – every week, or do it monthly – every month. Most of all do it the same way every time.
- The more you do it the better you get and the easier it will become.

