



# Wastewater Microbiology

Function & Evaluation

**Becky Hobden and Chris Flannery**

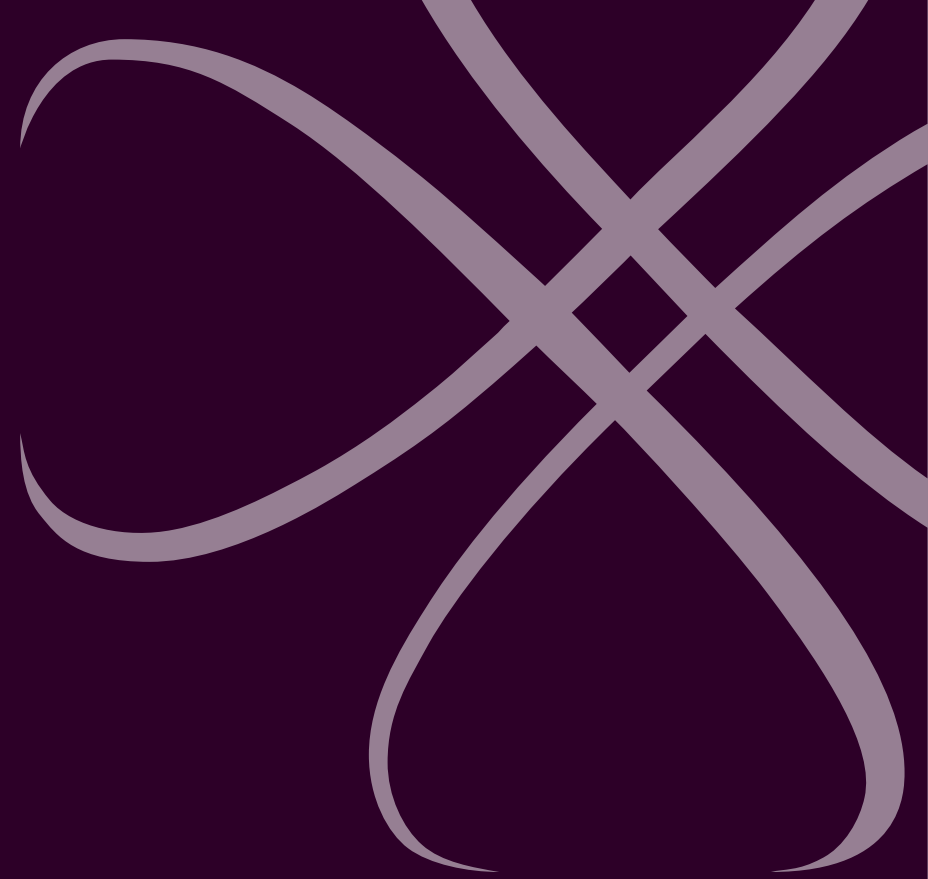
July 14, 2016



# Agenda

- 2:00 PM Introduction (5)
- 2:05 PM Wastewater Microbiology (25)
- 2:30 PM Wastewater Microscopy (30)
- 3:00 PM Break (30)
- 3:30 PM Live Demonstration of Performing Microexams (45)
- 4:15 PM Wrap Up & Questions (15)

# Introduction



# Introducing nature's problem solvers

Sometimes the greatest answers in life are found in its smallest components

Enzymes



Microorganisms



Catalyzing processes and building up or breaking down molecules



**Cleaner clothes**  
with less consumption of energy



**Better nutrition**  
with less food waste



**Green fuel**  
to reduce our dependence on oil



**Higher yields**  
and fewer pesticides



**Cleaner effluents**  
and more biogas

We also work with other proteins, biopolymers and related technologies

# Everywhere you need us



**Household Care**



**Food & Beverages**



**Bioenergy**



**Animal Health & Nutrition**



**Agriculture**



**Textile**



**Pulp & Paper**



**Leather**



**Wastewater Solutions**

# Our biological answers, near you



# Novozymes

Worlds largest producer of industrial enzymes and microorganisms.

- **Biofuels**
- **Detergents and cleaning products**
- **Agriculture**
- **Animal nutrition and aquaculture**
- **Food & beverages, (Juices, Beer, Wine, Cheese, Breads)**
- **Wastewater Solutions**

6500+ employees worldwide

150+ employees in Salem, VA

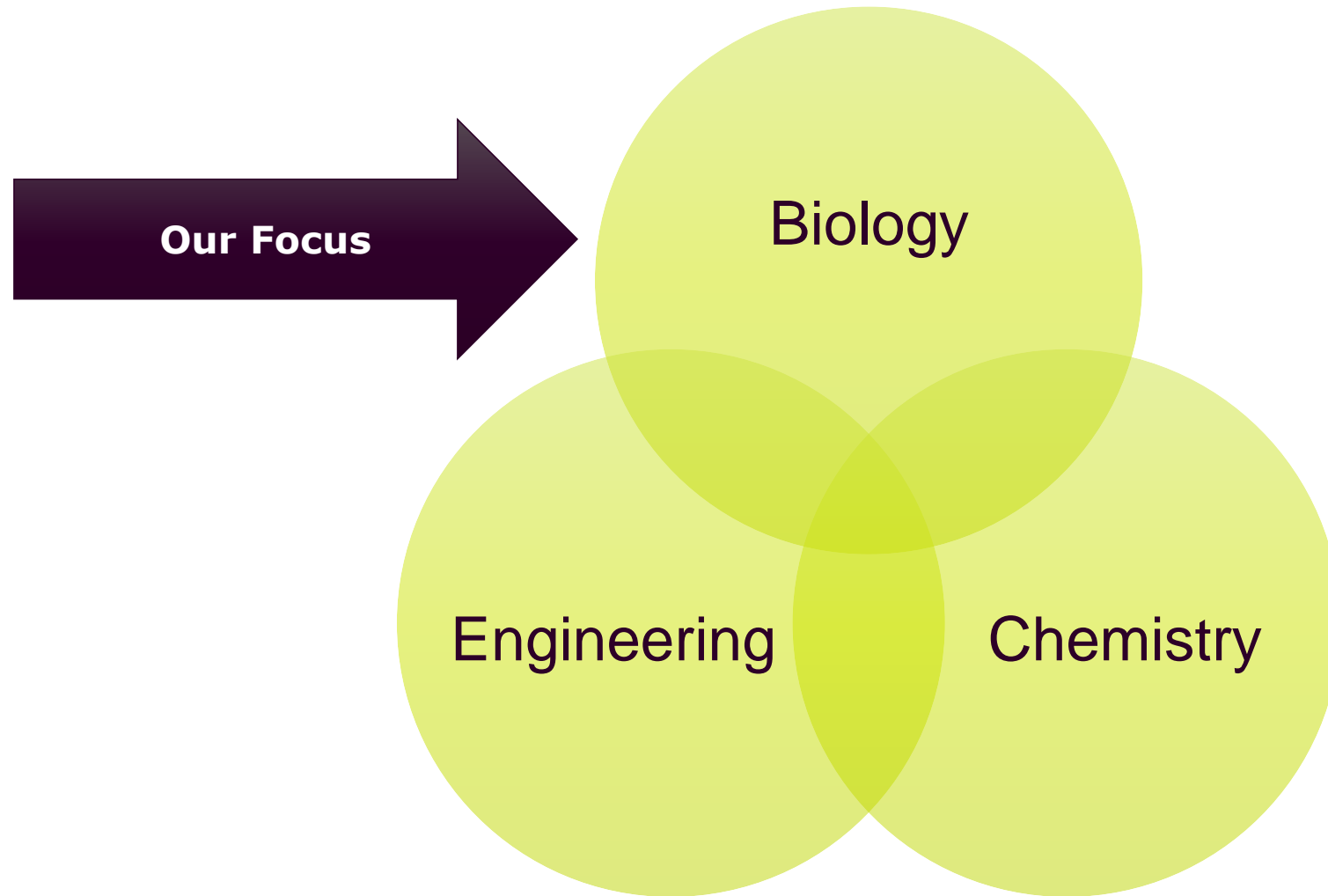


# Wastewater Microbiology





# Introduction



# Wastewater Microorganisms

The goal of biological wastewater treatment is to produce an environment in which microorganisms consume the maximum amount of organic substrate and produce clear effluent water.

To do this microorganisms must convert *soluble organic pollutants (BOD)* into *insoluble biomass (microorganisms)* which can be separated.



# What is a Microorganism?

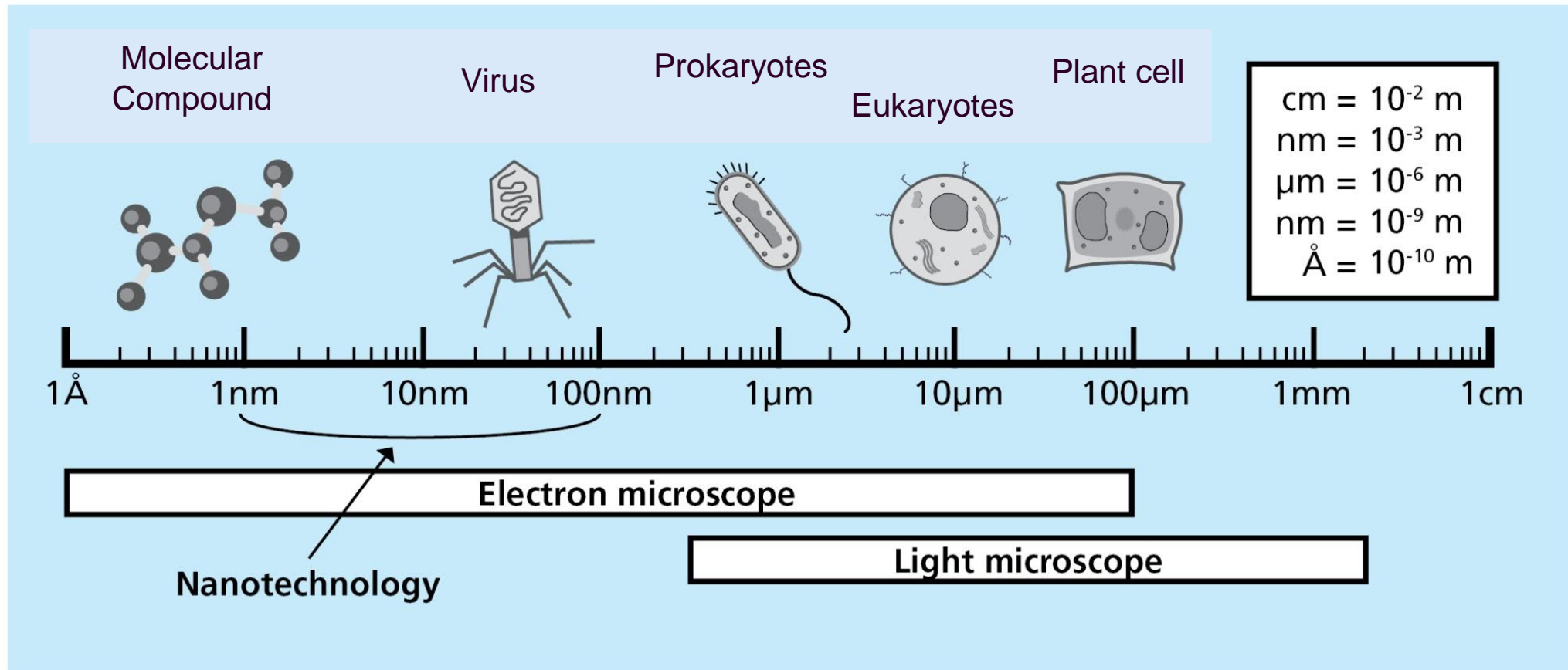
An ***organism*** is a living thing that can...

- grow
- reproduce
- react to its environment
- maintain homeostasis –  
temperature, pH, salinity, etc

What makes them “***micro-***”?



# Size of Microorganism – Typically 0.01 $\mu\text{m}$ to 1 mm

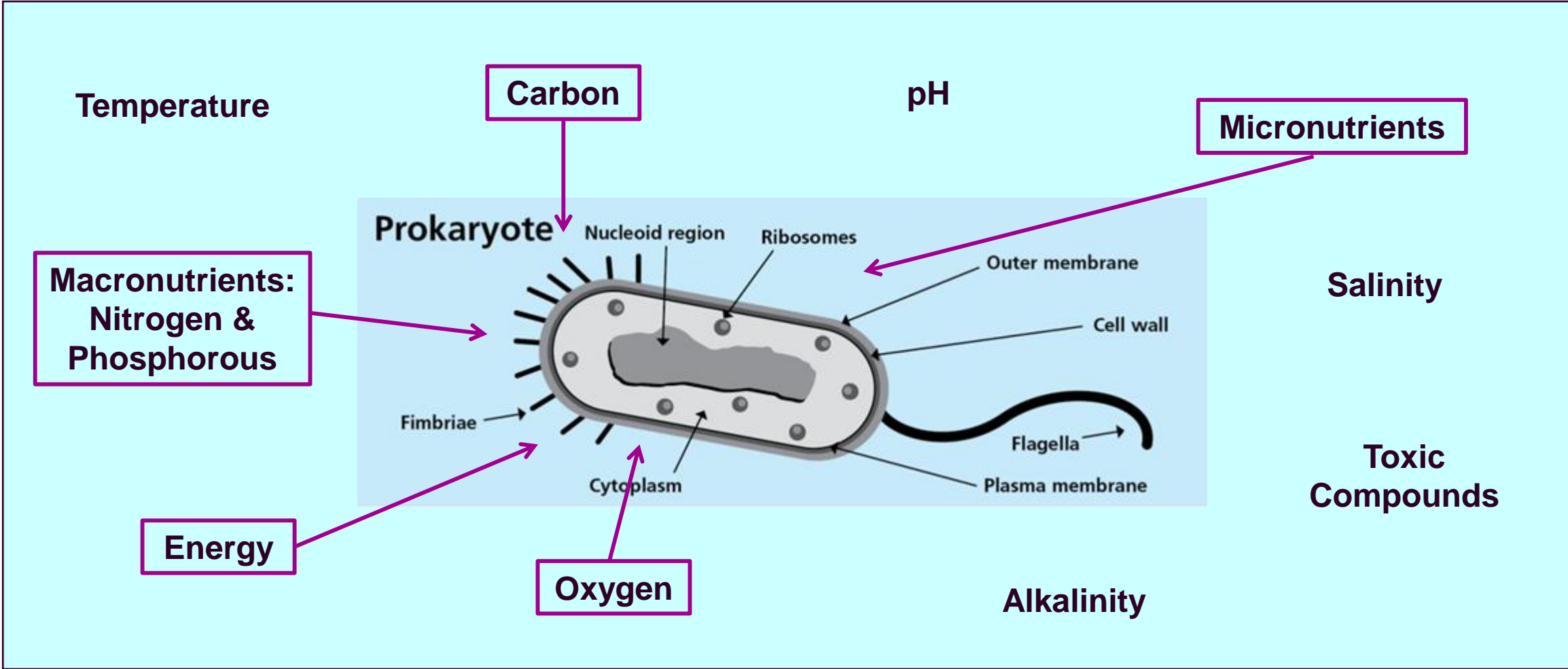




**What do microorganisms need to thrive?**



# Nutrients and Environment



# Composition of a Bacterial Cell

Element	Percentage by Weight (%)
Carbon	50
Oxygen	20
Nitrogen	14
Hydrogen	8
Phosphorous	3
Sulphur	1
Potassium	1
Sodium	1
Calcium	0.5
Magnesium	0.5
Chlorine	0.5
Iron	0.2
All others	0.3

Structure of Cell



Cellular water, organic cell material, e acceptor



Proteins, DNA, Enzymes



DNA, ATP, Cell Membrane



Vitamins or Co-metabolites  
(Micronutrients)

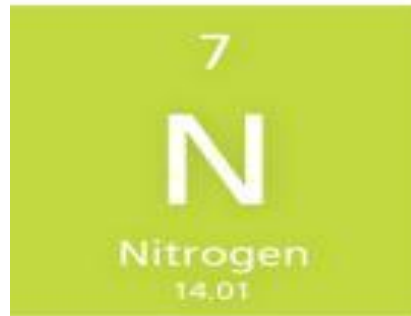


You are what you eat!

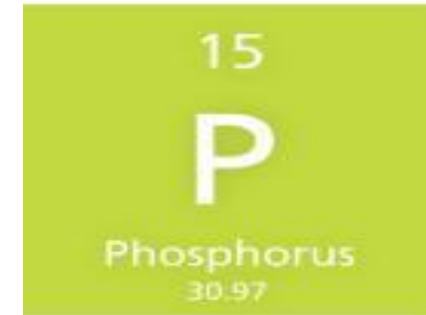
# Macronutrients



100



5



1

How do you test Nitrogen and Phosphorous?





# Oxygen

## Aerobic

- Exist only in environment of molecular oxygen.
- Nitrifiers – obligate aerobes

## Anaerobic

- Exist in an environment with no oxygen.
- Methanogens – obligate anaerobes

## Facultative

- Exist in an environment with or without oxygen.
- Anoxic Zones for denitrification



# ORP: Oxidation Reduction Potential

## Biochemical Reactions and Corresponding ORP Values

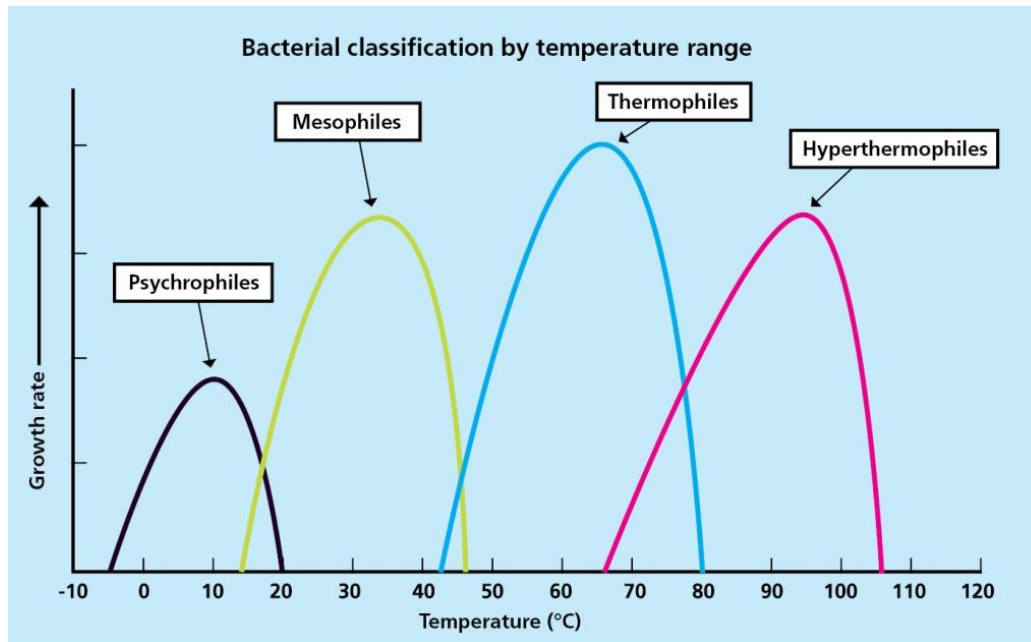
Biochemical Reaction	ORP, mV
Nitrification	+100 to +350
cBOD degradation with free molecular oxygen	+50 to +250
Biological phosphorus removal	+25 to +250
Denitrification	+50 to -50
Sulfide (H <sub>2</sub> S) formation	-50 to -250
Biological phosphorus release	-100 to -250
Acid formation (fermentation)	-100 to -225
Methane production	-175 to -400

# Temperature

In general, warmer environments have more microbial activity.

**Wastewater - focus on the mesophiles.**  
**Anaerobic treatment - can be thermophilic.**

Mesophilic bacteria can adapt to a wide range... must have time to acclimate.



	Temperature (°C)		
Group	Minimum	Optimum	Maximum
Thermophiles	40 - 50	55 - 75	60 - 80
Mesophiles	10 - 15	30 - 45	35 - 47
Psychrotrophs	-5 - 5	25 - 30	30 - 35
Psychrophiles (obligate)	-5 - 5	15 - 18	19 - 22

# pH

Each microorganism has a pH optimum and range.

- Natural habitats usually are between 5 and 9.
- Few organisms are able to grow at a pH below 2 and above 10.
- Wastewater applications are optimum at 6 to 8.

pH changes can be tolerated... but need acclimation.

Group	pH Range
Acidophiles	<1 to 4.5
Neutrophiles	5.5 to 8.5
Alkalophiles	7.5 to 11.5





# Alkalinity – NOT the same as pH

The ability of a solution to neutralize (buffer) acids typically with carbonate or bicarbonate

Important in wastewater microbial processes:

- Nitrification:
  - 7.1 ppm  $\text{CaCO}_3$  for each ppm of  $\text{NH}_4$  reduced
- Anaerobic processes generate acids (VFAs) during acidogenesis and acetogenesis:
  - Approx. 3/1 ratio of VFA/Alkalinity

# Microorganism Life Cycle

## Lag Phase

- Microbes are adapting to environment

## Log Growth Phase

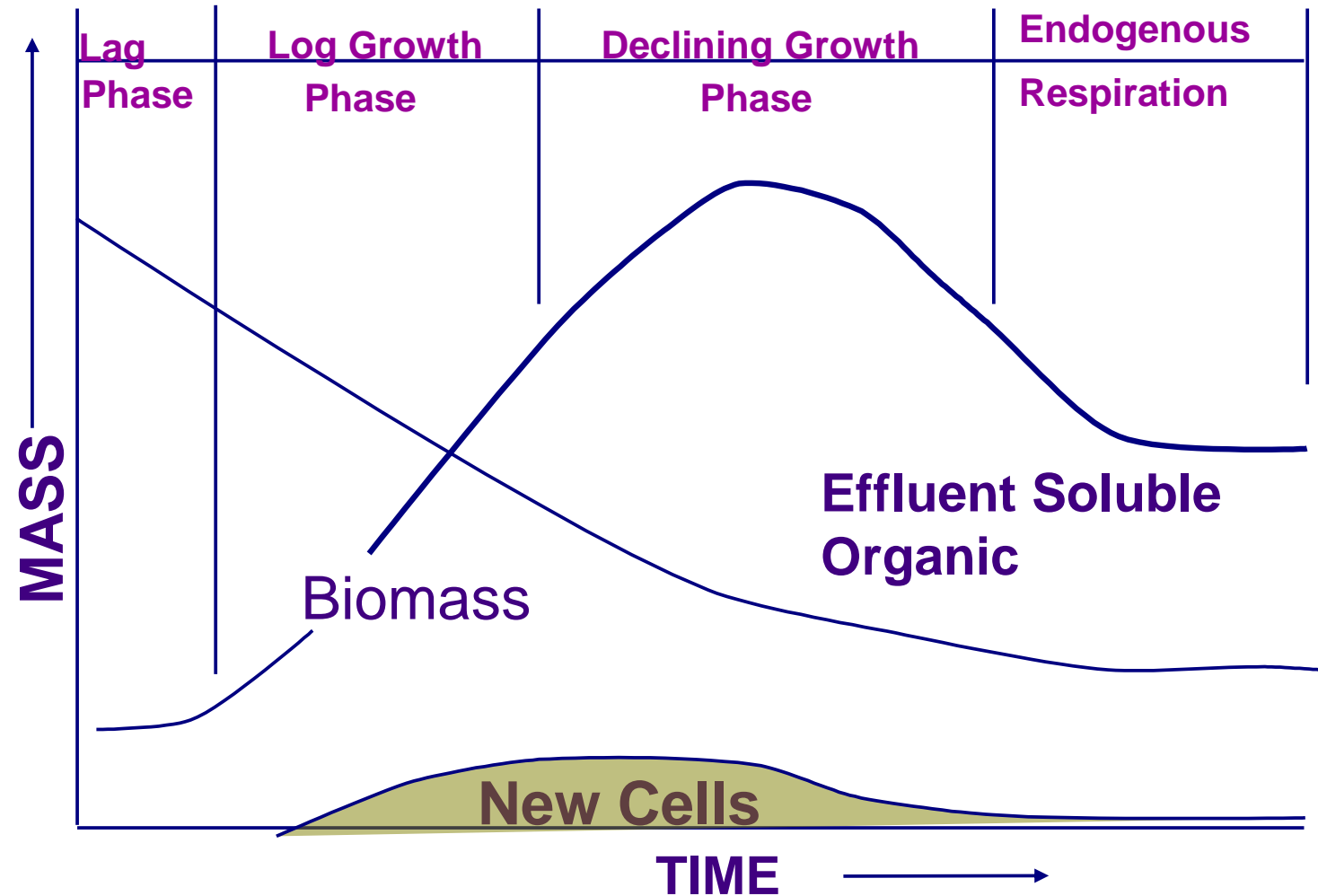
- Constant, exponential growth

## Declining Growth Phase

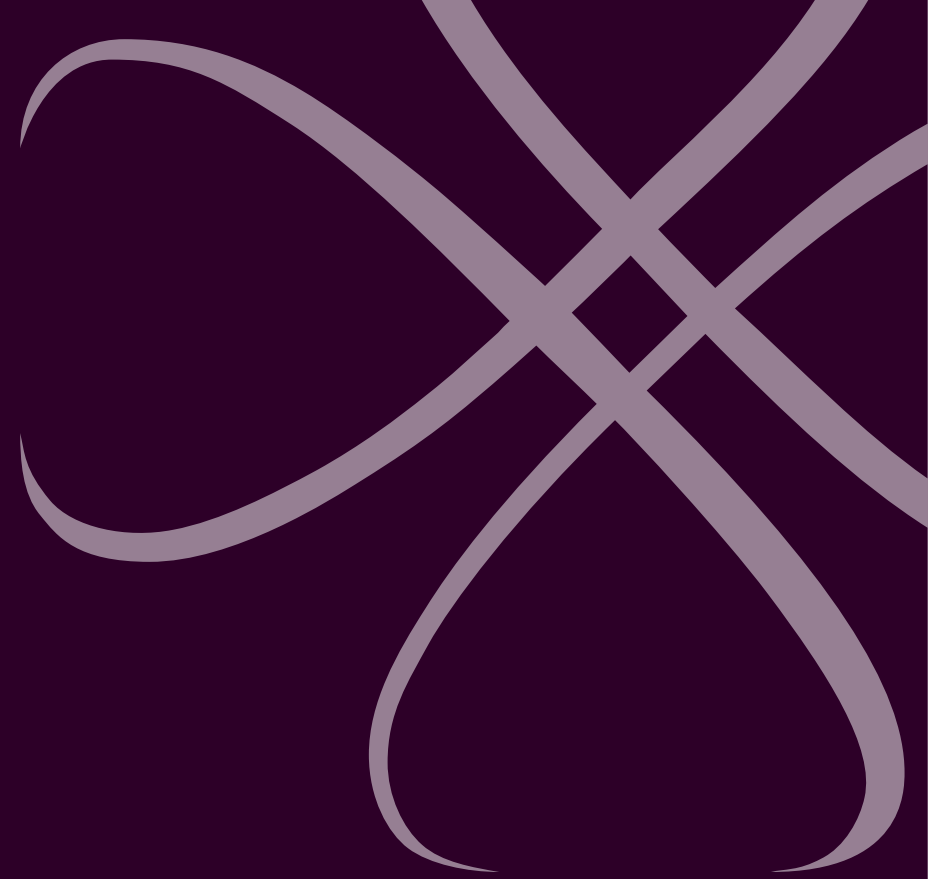
- Growth rate declines (food, nutrients, electron acceptors)

## Endogenous Phase

- Death phase (limitation of resources to sustain viable population)



# Wastewater Microscopy



# Why is wastewater microscopy important?

Critical for monitoring and troubleshooting

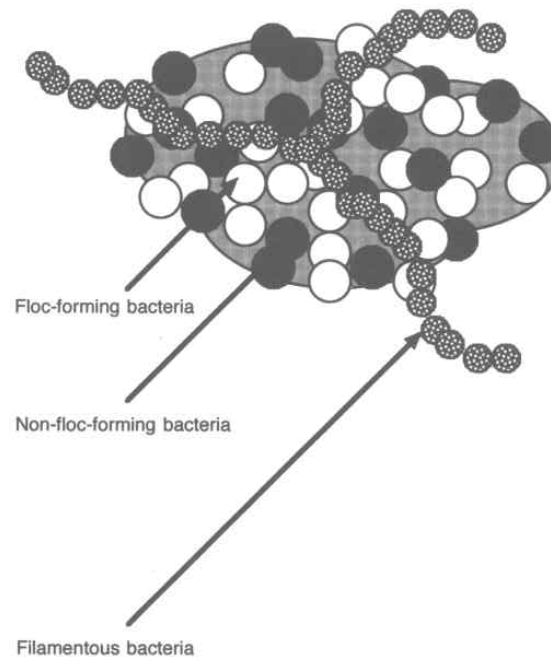
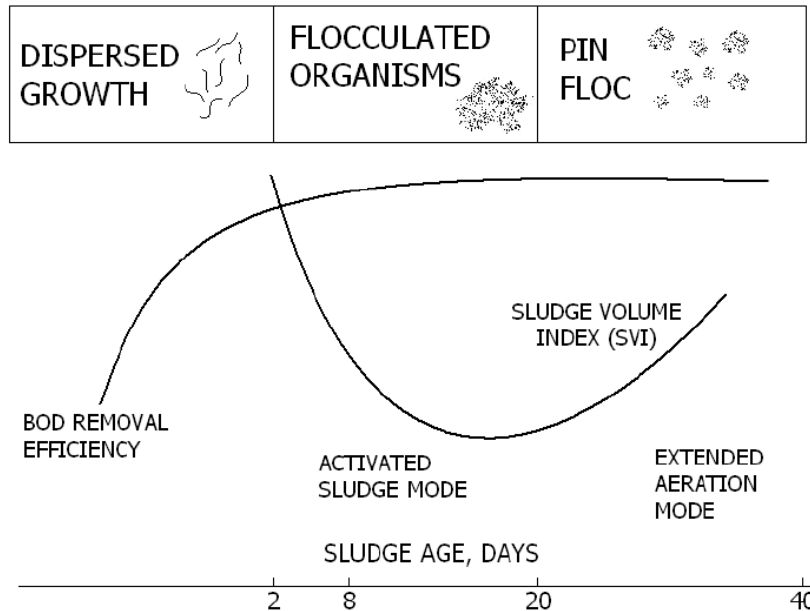
Make observations and detect changes

P/M & filament ID to troubleshoot settling and plant performance issues

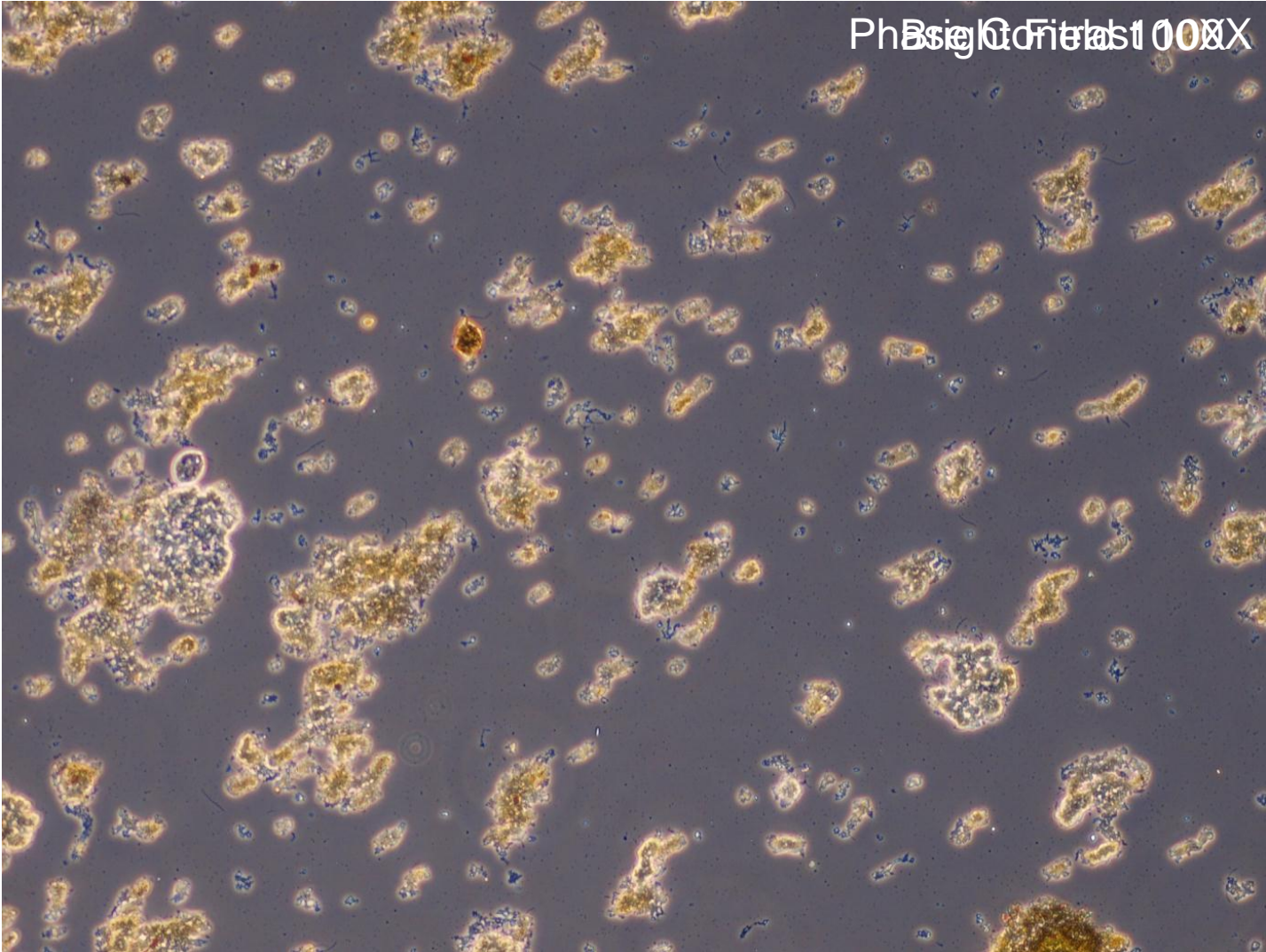


# What can you do with microscopy?

1. You can evaluate floc formation.
2. You can evaluate filamentous bacteria content and bulking
3. You can evaluate the higher life forms
4. You can evaluate the bulk water

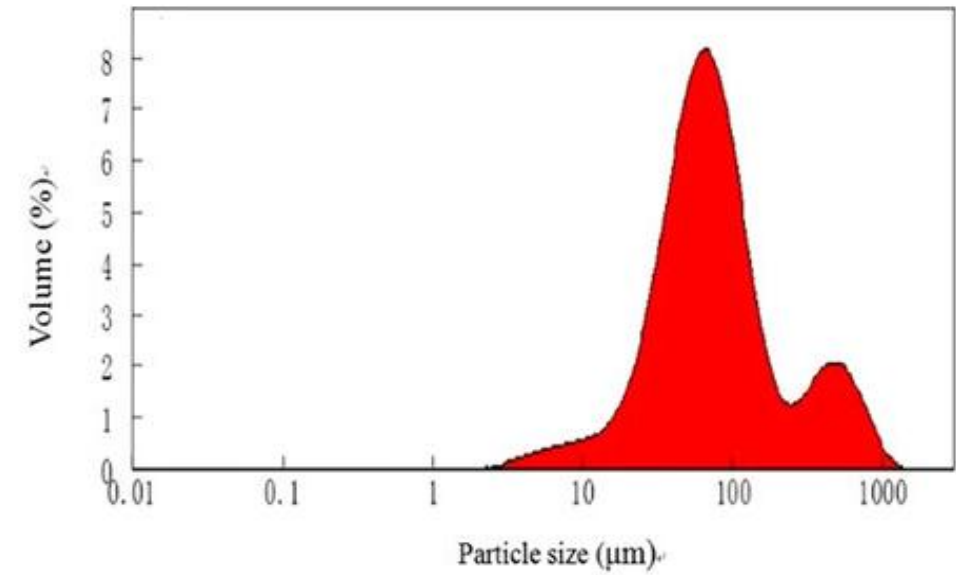


# Phase contrast

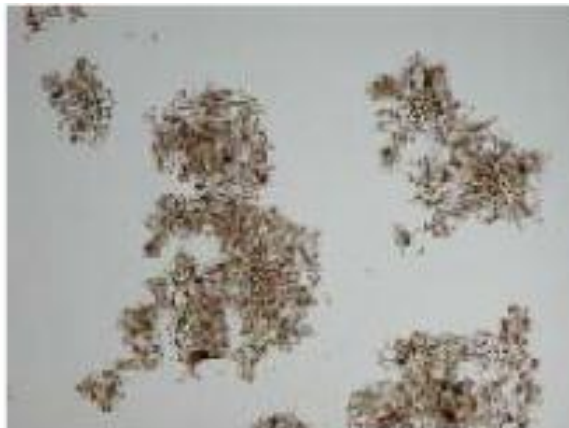


# Floc Characterization

- Size, shape, strength/density
- Dispersed growth
- Open floc formation
- Interfloc bridging
- India ink reverse stain
  - Levels of EPS (loosely bound or tightly bound)
  - Zoogloal growth



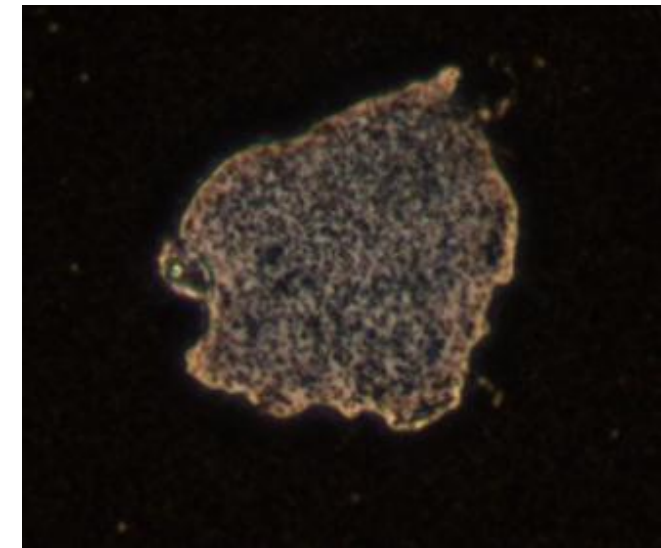
(a)



(b)



(c)





# Filamentous Bacteria

String or threadlike bacteria

Chains of cells which can extend from the floc, grow within the floc, or even free in the bulk water.

Prevent effective settling by interfering with floc formation (bulking).

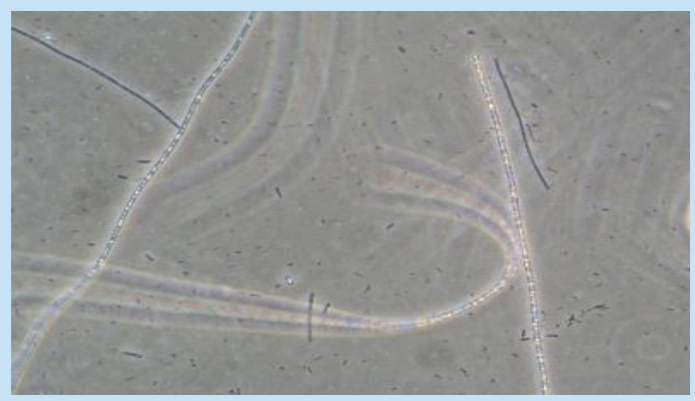
Can be helpful in small quantities, acting as a backbone for floc to form.

We care about:  
**IDENTIFICATION AND  
ABUNDANCE**

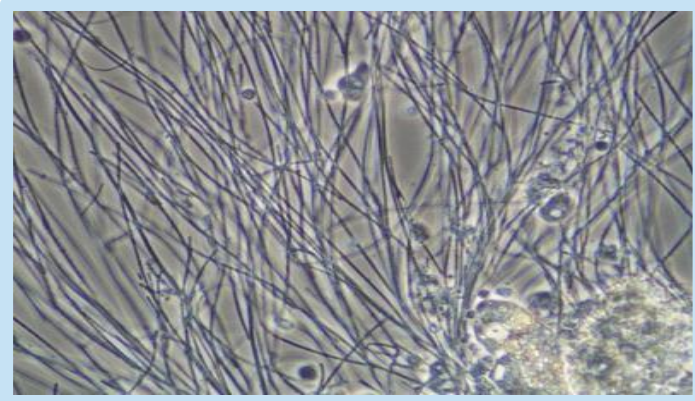


# Examples of Filamentous Bacteria

*Beggiatoa*



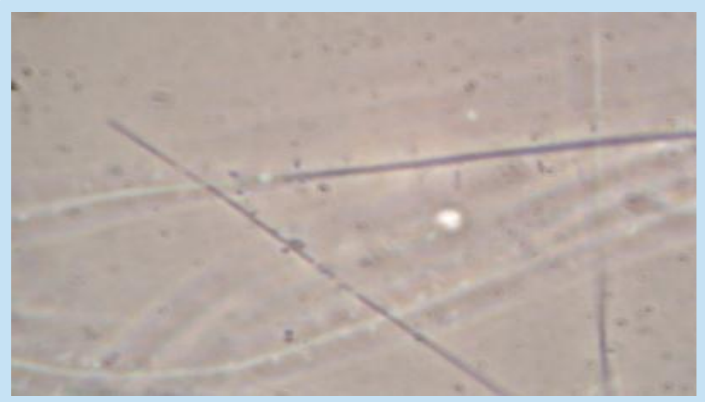
*Microthrix parvicella*



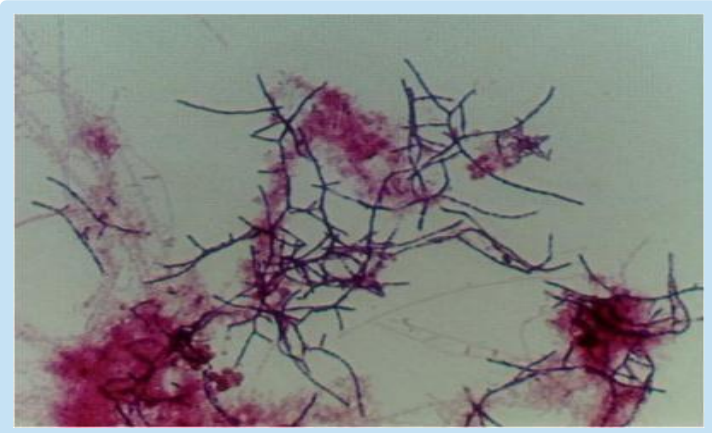
*S. natans*



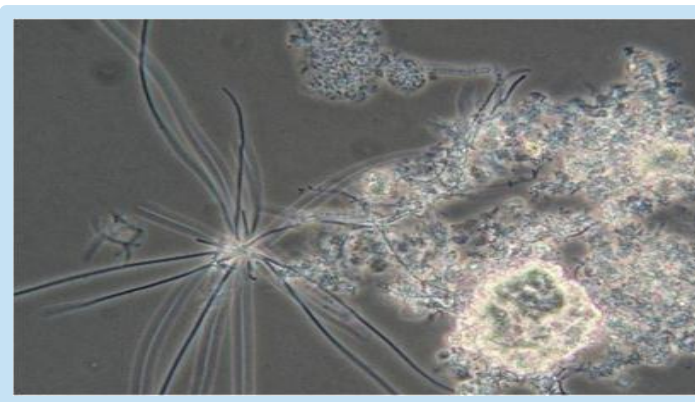
*H. hydrossis*



*Nocardia*

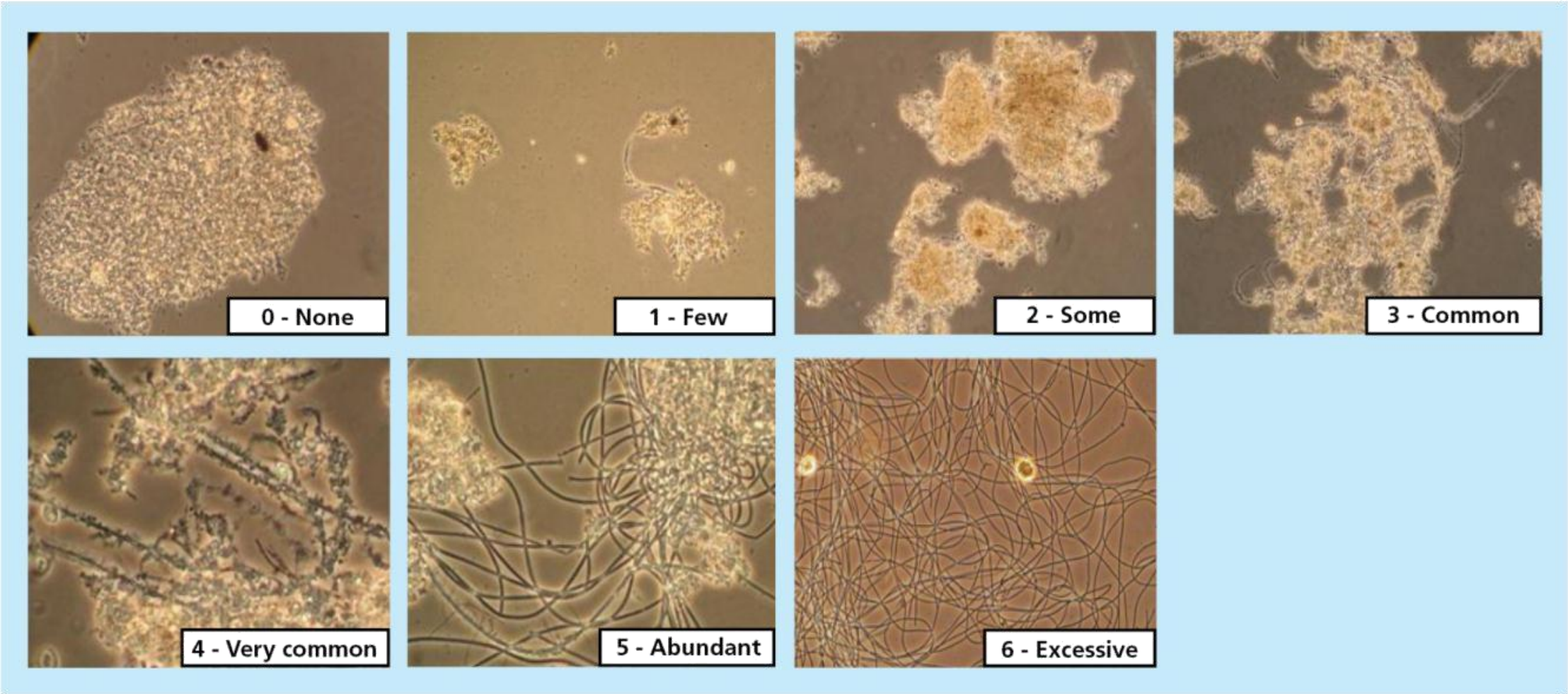


*Thiothrix*





# Filamentous Index





# Filaments and associated causes

Manual on the Causes and Control of Activated Sludge Bulking, Foaming, and Other Solids Separation Problems 3<sup>rd</sup> edition  
Jenkins, Richard, Daigger Lewis Publishers

Low Dissolved oxygen      Sphaerotilus natans, Type 1701, Haliscomenobacter hydrosis

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Low F/M      Type 0041, Type 0675, Type 1851, Type 0803

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Septicity      Type 021N, Thiothrix I&II, Type 0914, Type 0411, Type 0961, Type 0581, Type 0092, Nostocoida limicola I, II, and III

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Oil and Grease      Nocardia sp., Microthrix parvicella, Type 1863

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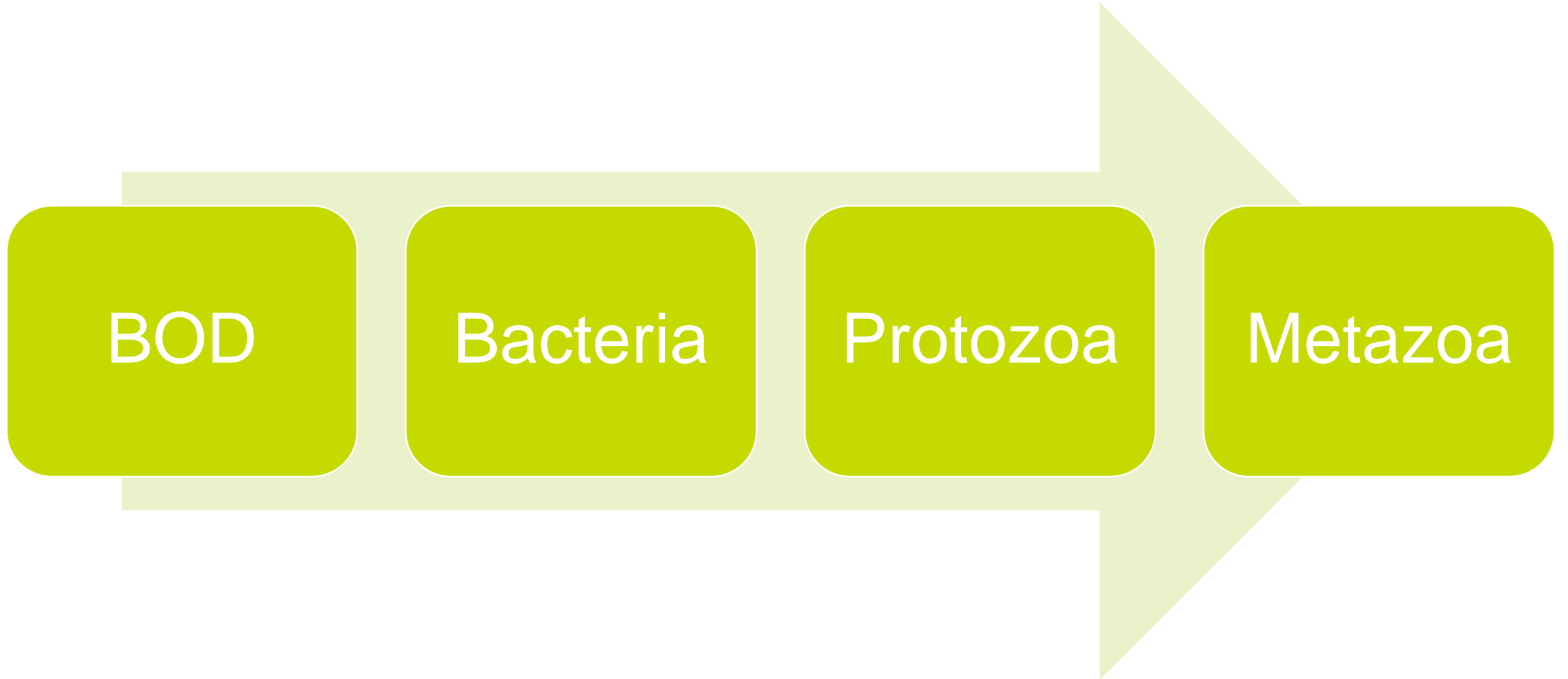
Nutrient Deficiency      Type 021N, Thiothrix I and II, S. natans, N. limicola III, H. hydrosis

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Low pH      Algae

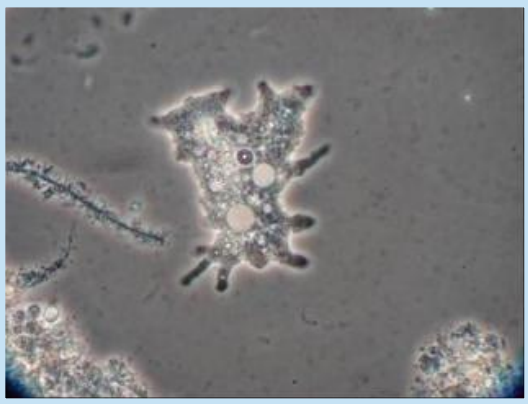
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# Higher Life



# Protozoa (single celled)

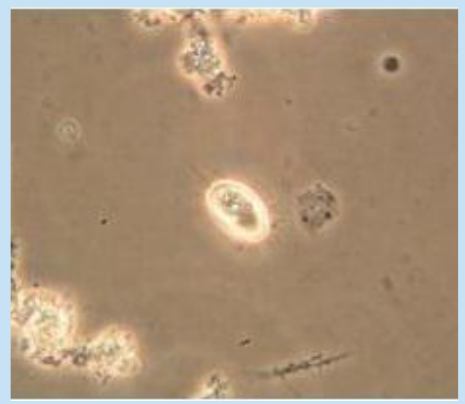
Amoeba



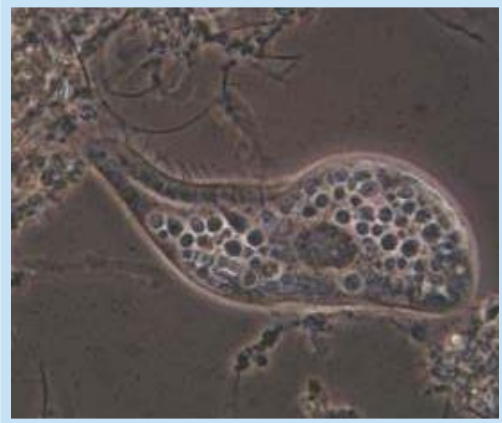
Flagellates



Free-Swimming Ciliates



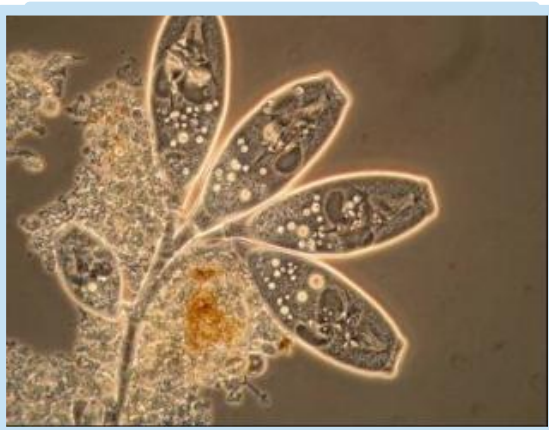
Carnivorous Ciliates



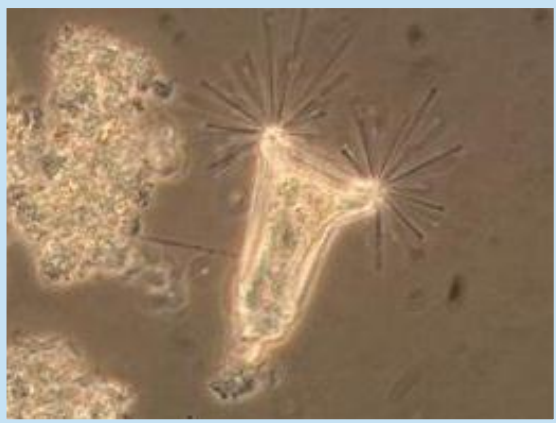
Crawling Ciliates



Stalked Ciliates



Suctoria



# Metazoa (many celled)

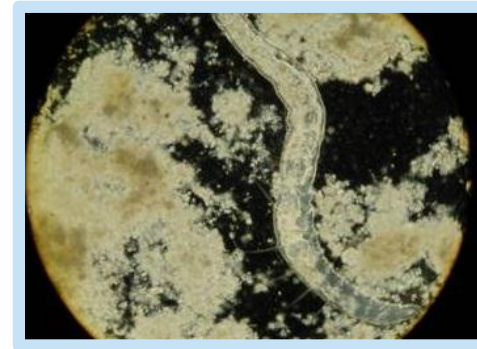
Rotifers



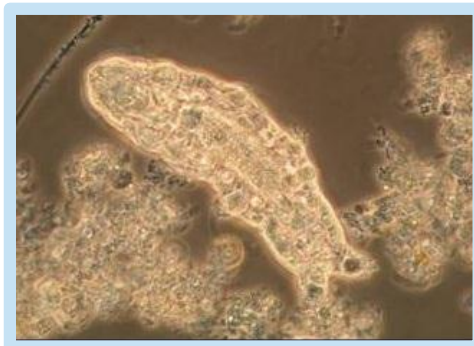
Gastrotrichs



Aeolosoma Worms



Tardigrades

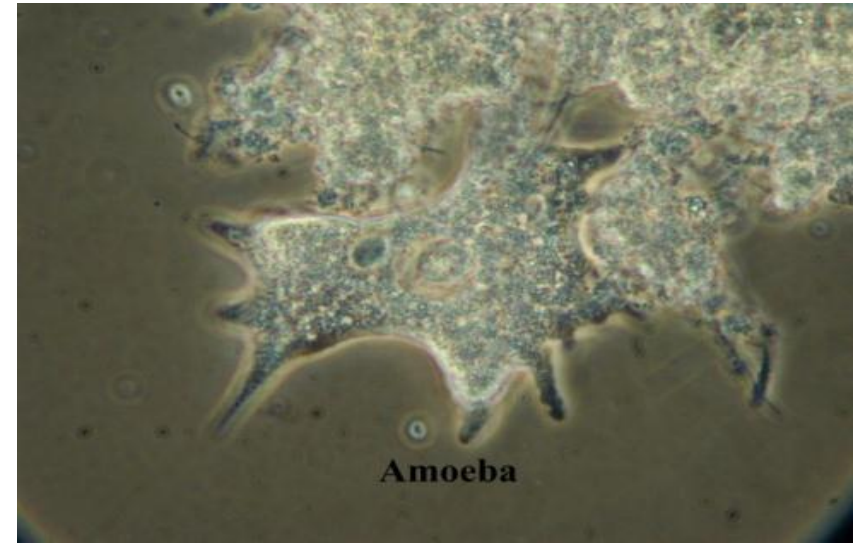


Nematodes

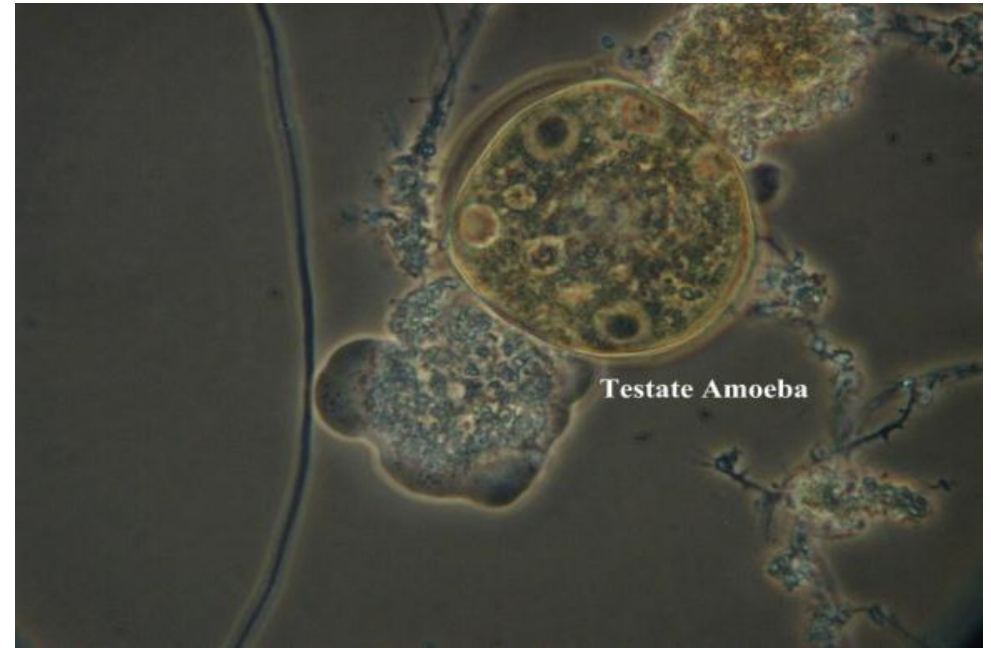




# Amoeba



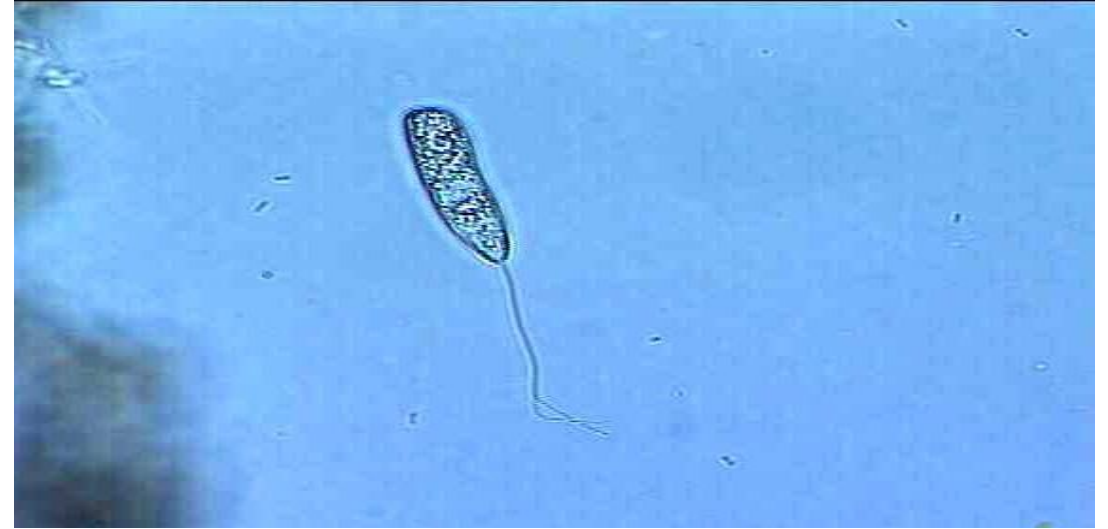
- Slowly mobile or not
- Present at start-up
- May indicate a recent upset event
- Grow well on particulate organic matter
- Can tolerate low DO



# Flagellates



- Length = 10 – 25  $\mu\text{m}$
- Motile by a whip-like tail
- Feed on soluble organic matter
- May indicate upset conditions
- Proceed amoeba at after start-up



- Some species (not shown) are much larger.
- Feed on bacteria and particulate matter
- Not necessarily associated with upsets

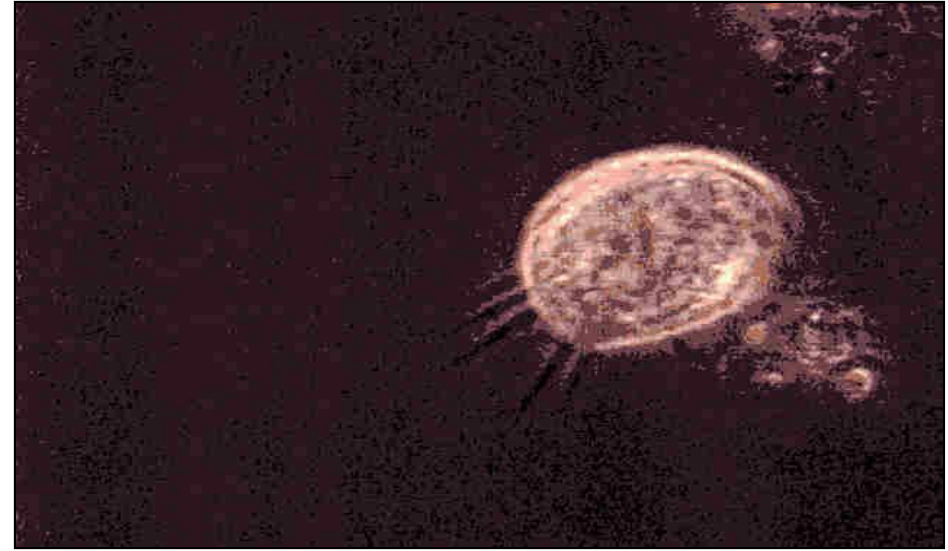


# Swimming Ciliates



- Length = 50 – 150  $\mu\text{m}$
- Motile by hair-like cilia
- Feed off of dispersed bacteria and floc particles
- Usually occur under conditions of good floc formation
- Indicate good activated sludge operation

# Crawling Ciliates

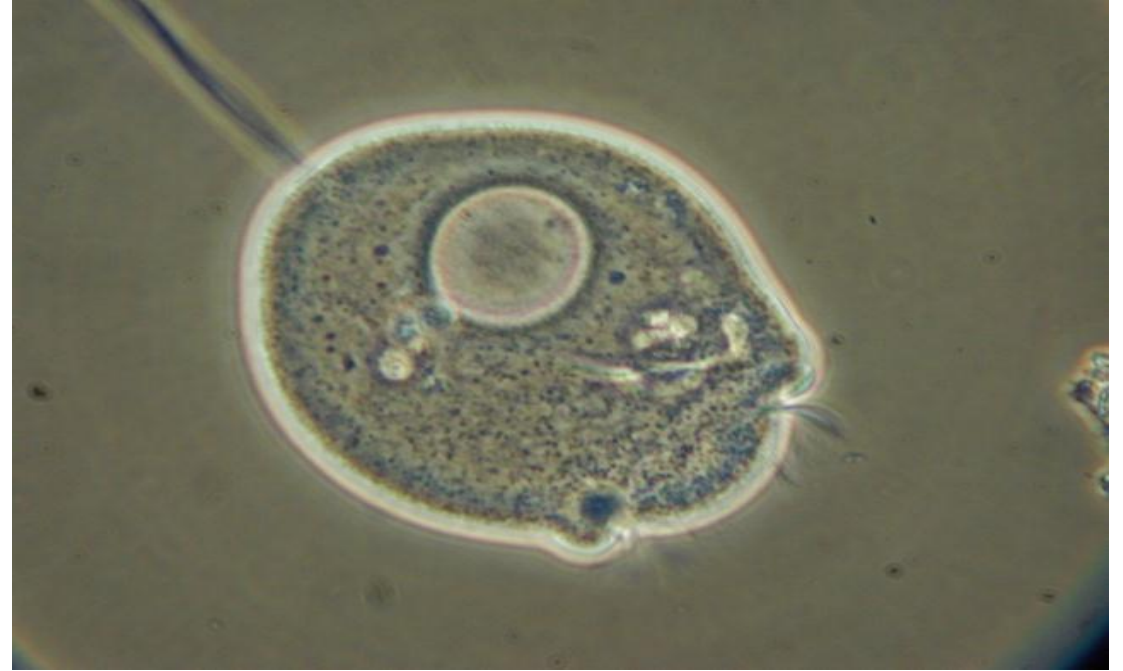


- Length – 25 – 60  $\mu\text{m}$
- Motile by “crawling” over floc particles
- Feed off of floc particles
- Usually occur under conditions of good floc formation
- Indicate good activated sludge operation

# Stalked Ciliates



- Length 50 – 100  $\mu\text{m}$
- Found attached to flocs by a stalk
- Some may contract.
- Indicate stable activated sludge operation
- Colonial forms may indicate at long MCRT.





# Rotifers



- Length 100 – 500  $\mu\text{m}$
- More complex than protozoa -- multi-celled with structural zones
- Motile by a contractile “foot”
- Usually occur at higher MCRTs

# Gastrotrichs



60  $\mu\text{m}$  to 3 mm long

Cilia around the body

2 posterior "feet"

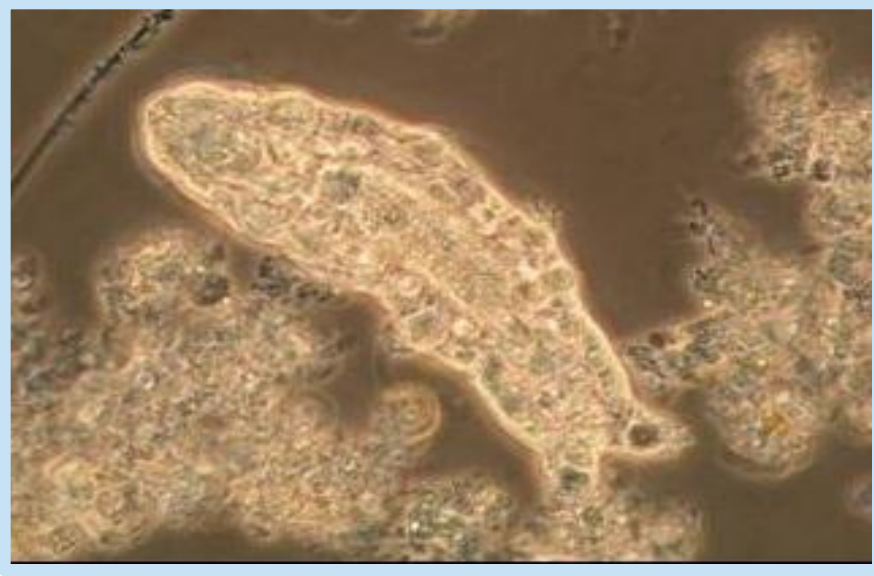
Detritivore, feed on just about anything (organics, bacteria, protozoa)

Not very indicative of plant conditions



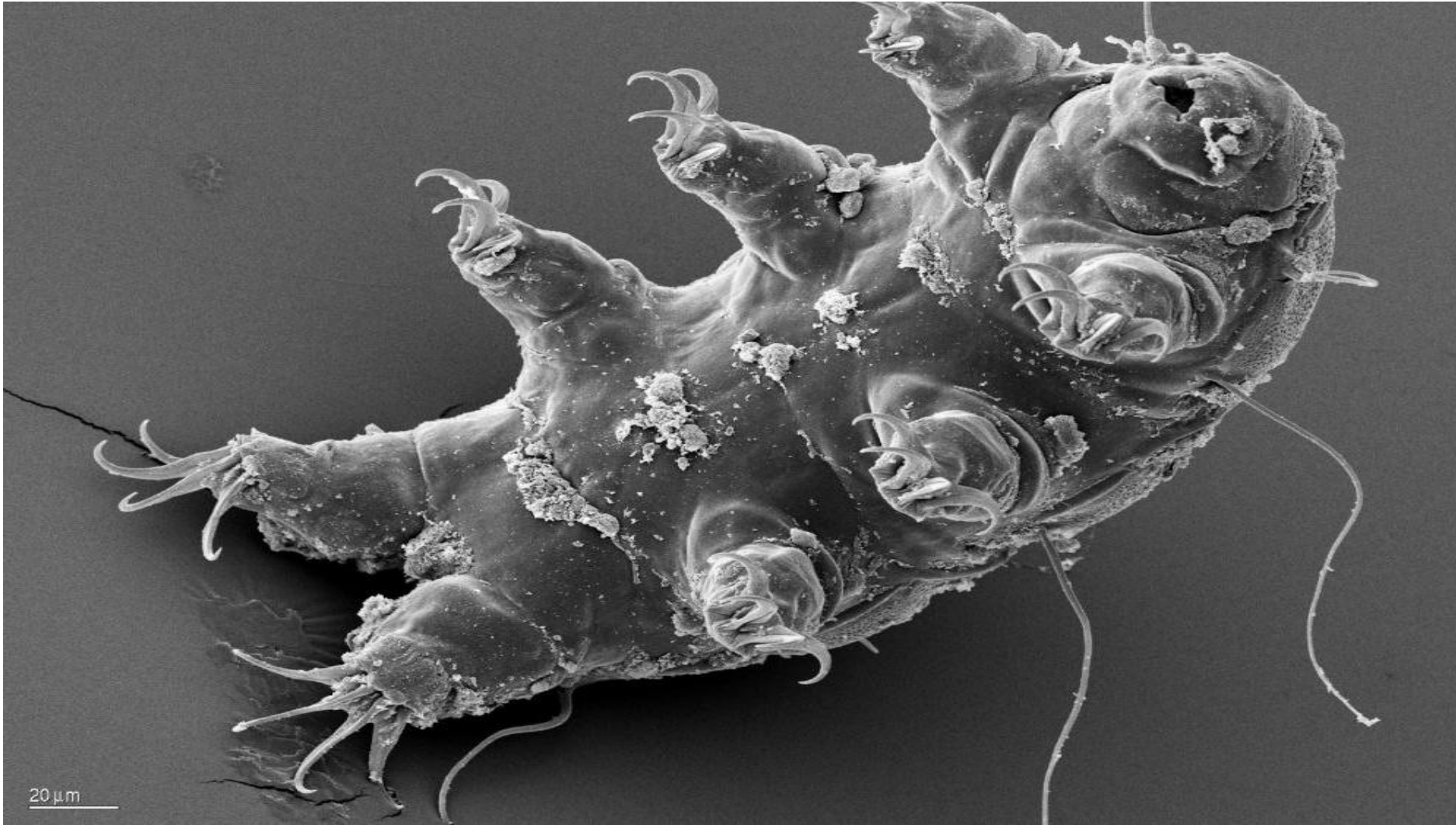
*Vago, G. 2012*

# Tardigrades (Water Bears)



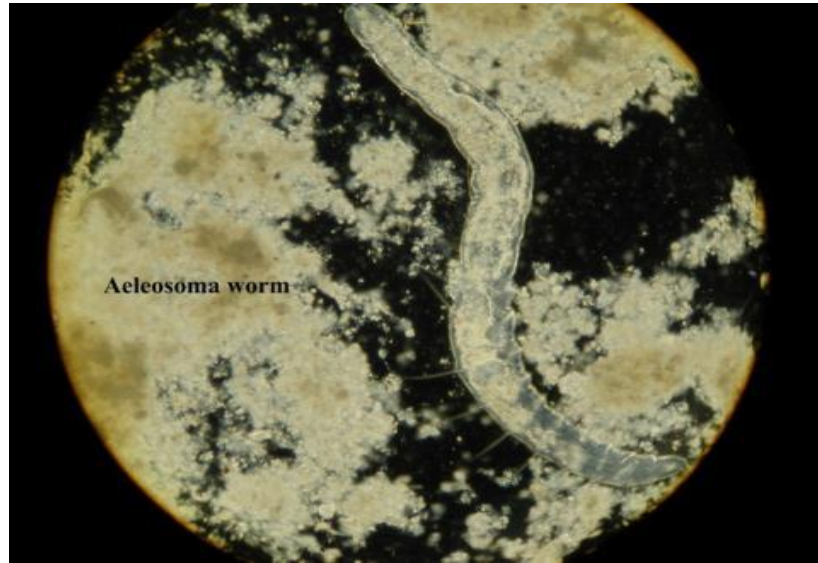
- Eight legs, with claws
- Healthy diversity
- Stable systems
- Longer sludge age
- Carnivore, feeds on protozoa and other metazoa





“Tolerance of Anhydrobiotic Eggs of the Tardigrade *Ramazzottius varieornatus* to Extreme Environments.” By Daiki D. Horikawa et al. *Astrobiology*, published online April 10, 2012.

# Bristle Worms



- Segmented
- Red-pink spots, red-pink sludge
- Tolerate low DO
- **Low F/M**
- **Old sludge**
- Seen with high nitrates

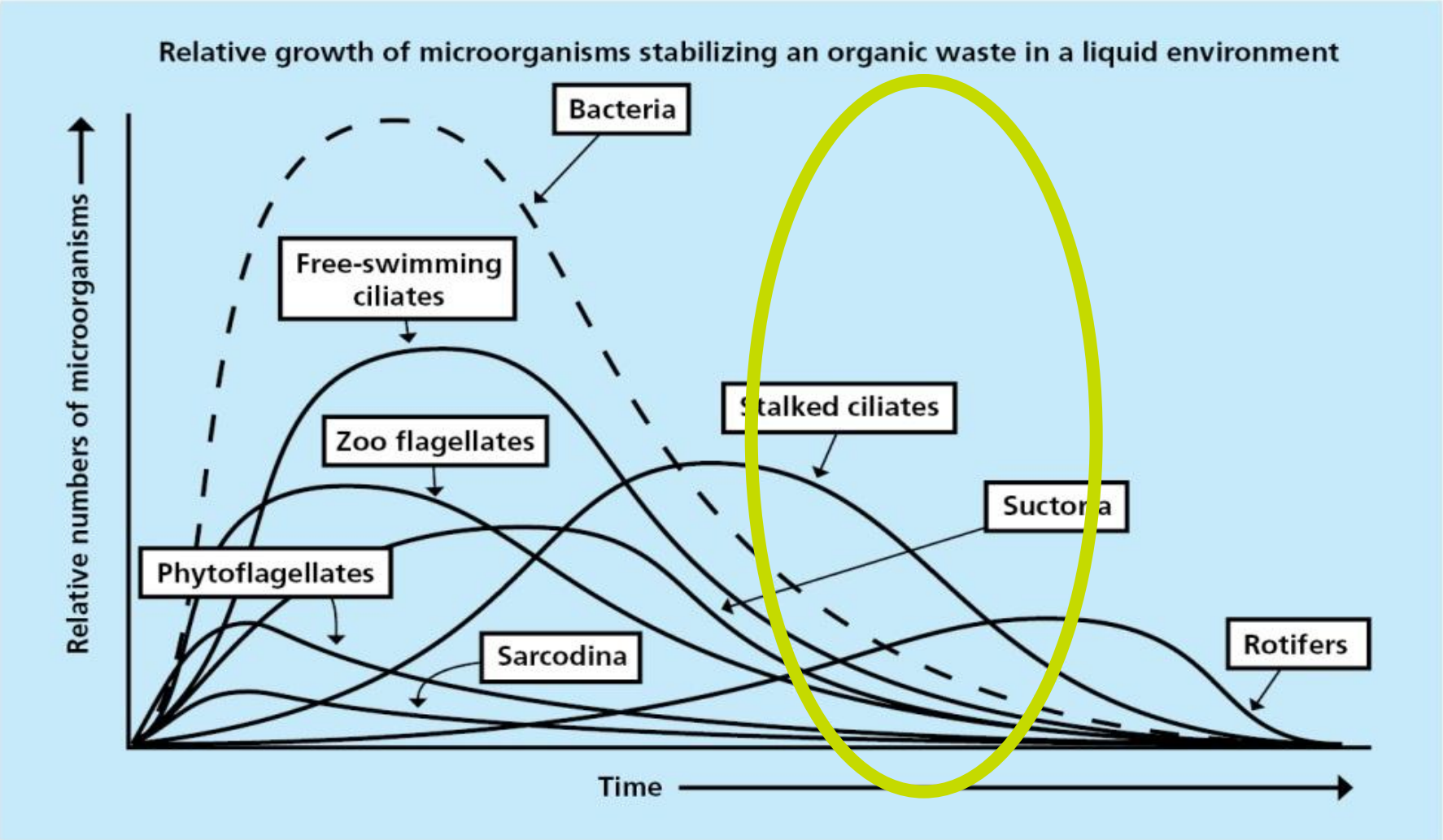
# Nematodes



- Detrivores
- More aerobic
- **Low F/M**
- **Old sludge**



# Biology of Wastewater Treatment

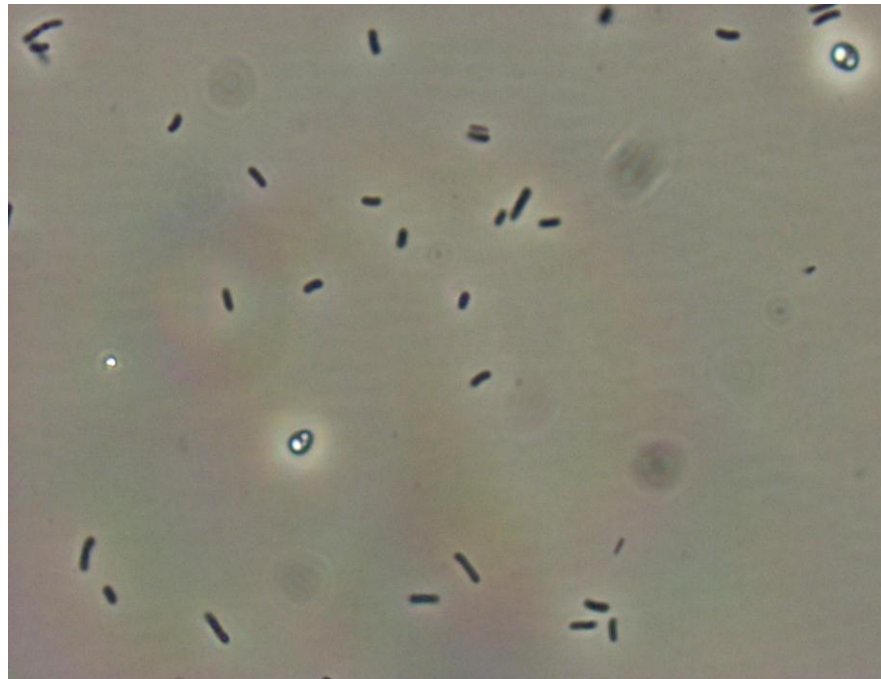
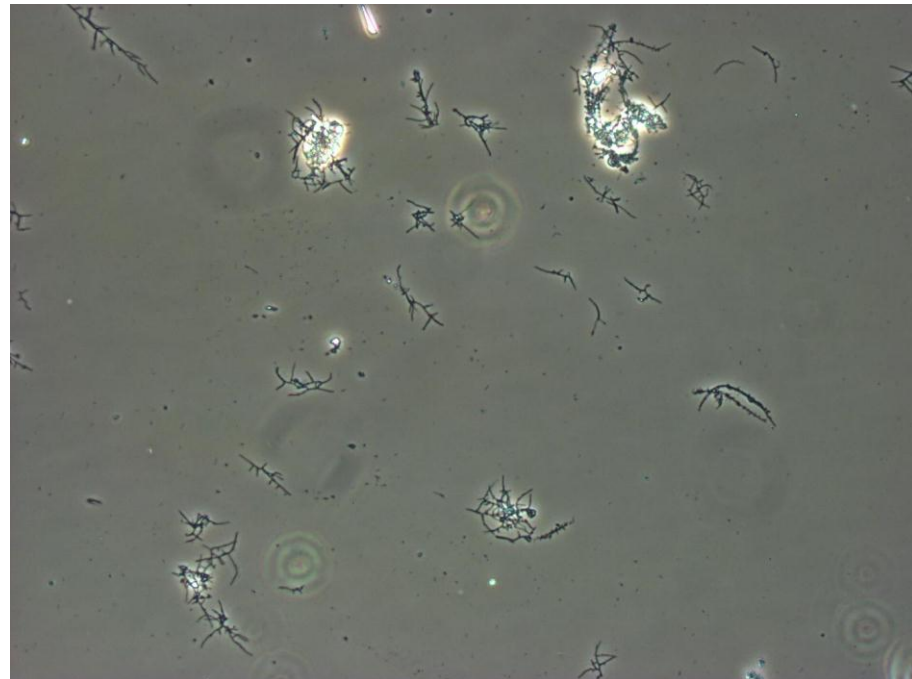
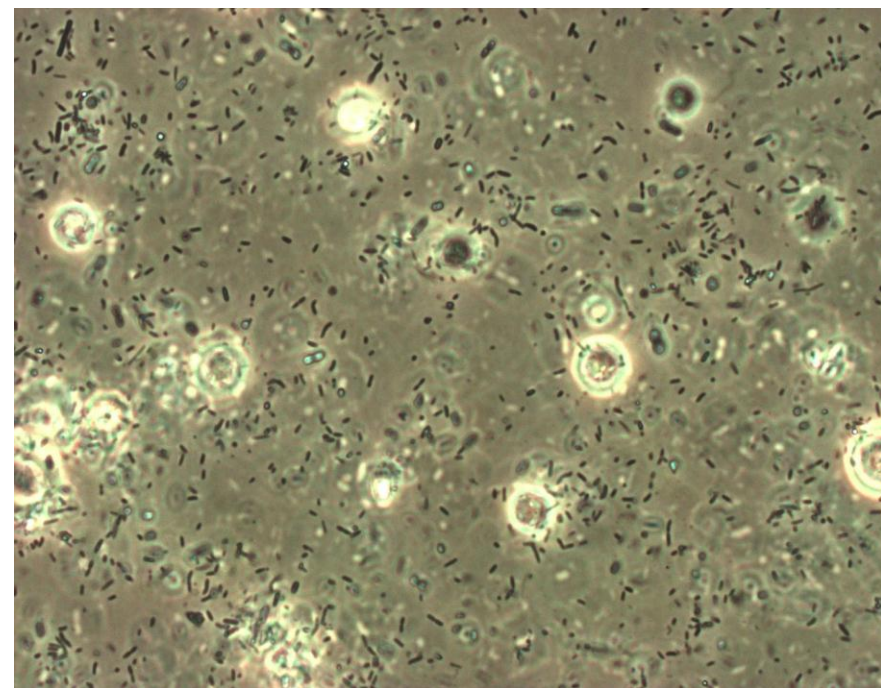
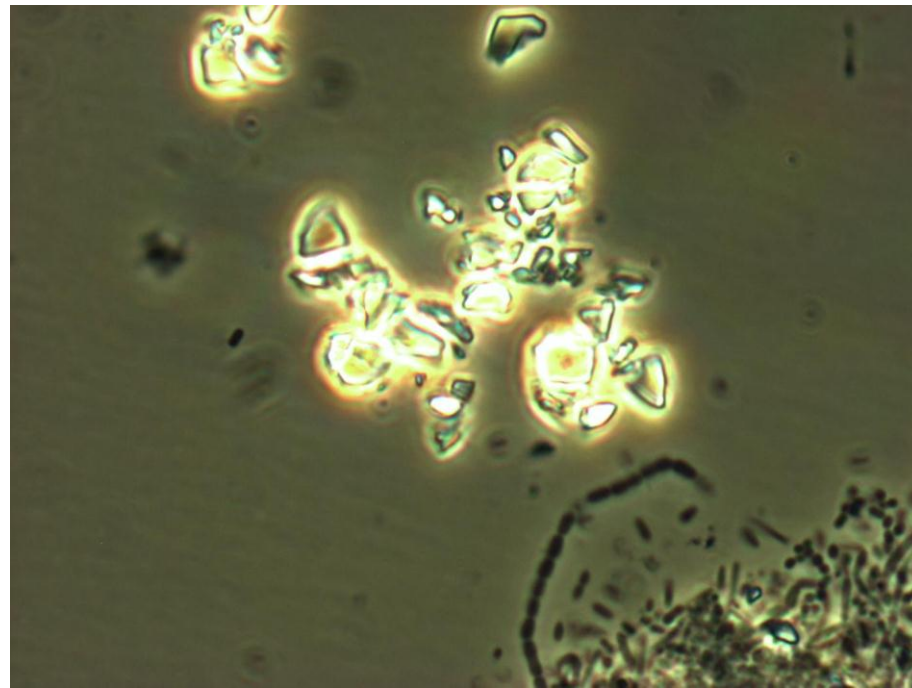


# Bulk Water

The water around the floc.

Equals secondary clarifier effluent

Look for non-floc bacteria, debris, particulates, filaments, etc as they may become effluent TSS.





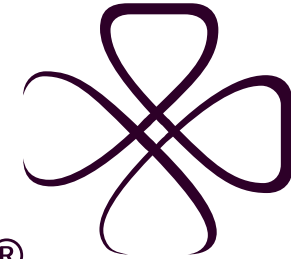
# Live Microscopic Examinations





# Questions

novozymes®



**Rethink Tomorrow**

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