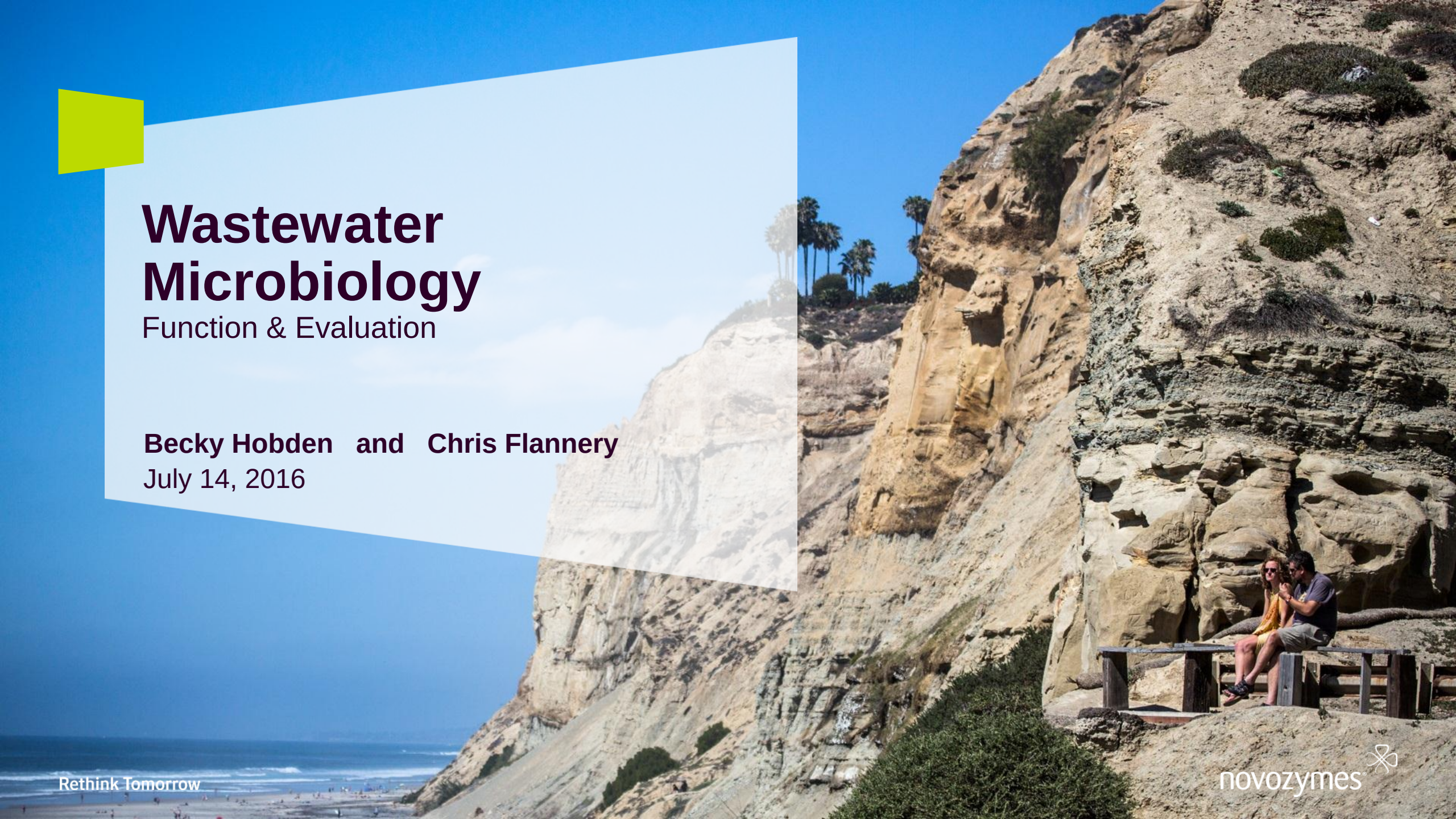
Wastewater Microbiology

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)

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Introduction

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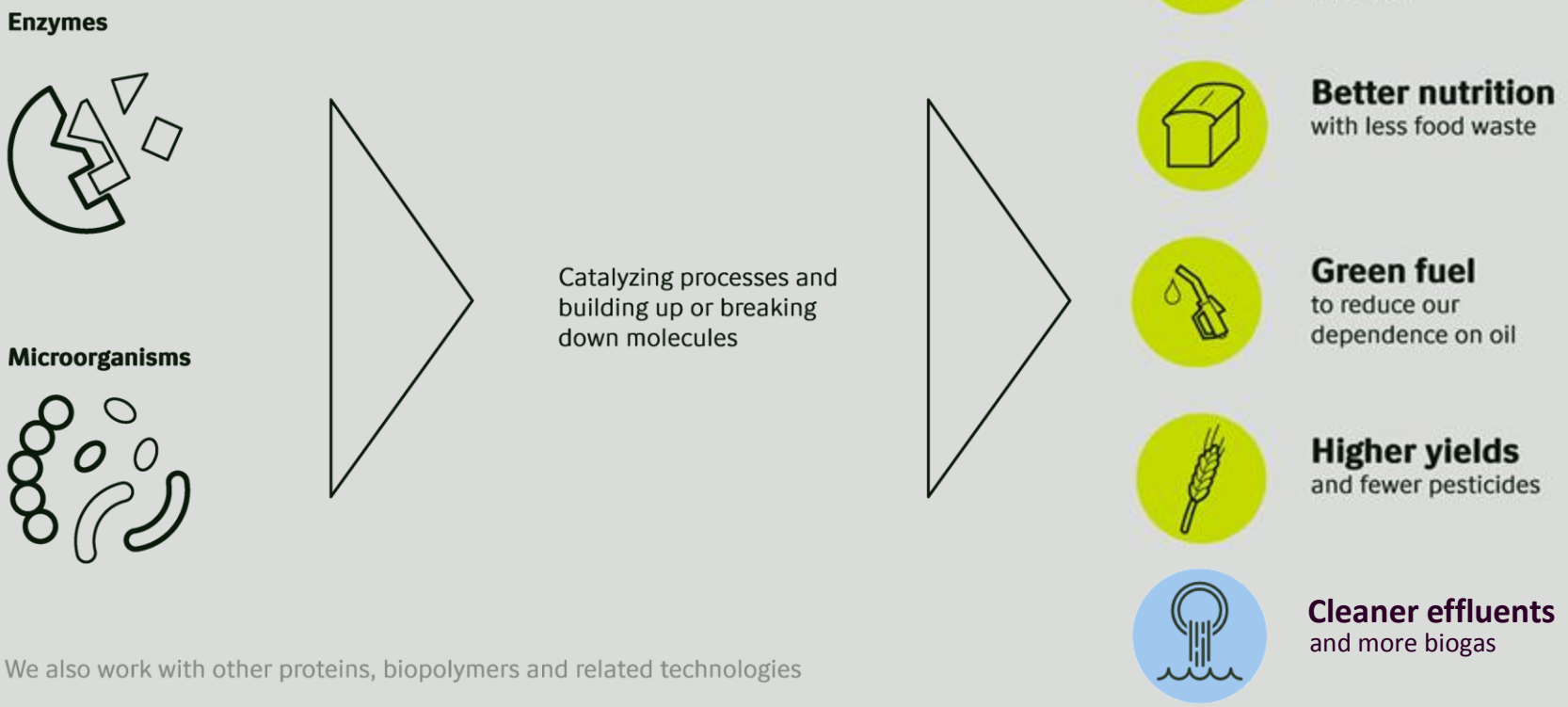
Introducing nature's problem solvers

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



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Worlds largest producer of industrial enzymes and microorganisms.

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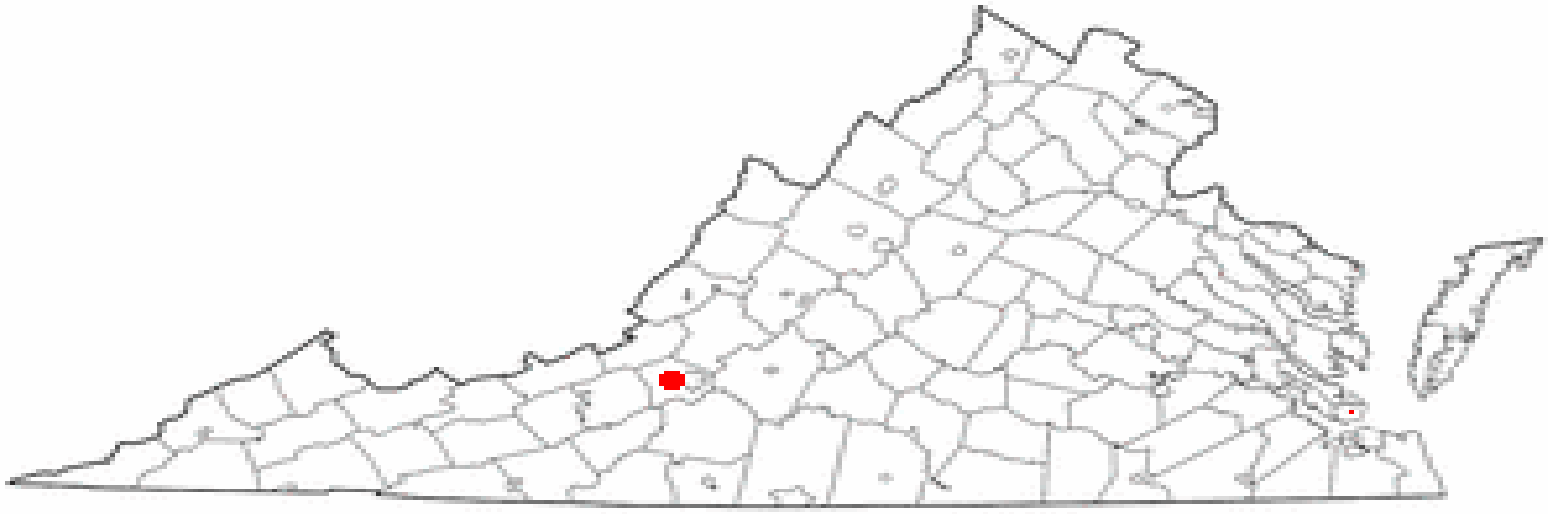
- **Wastewater Solutions**

6500+ employees worldwide

150+ employees in Salem, VA



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Wastewater Microbiology

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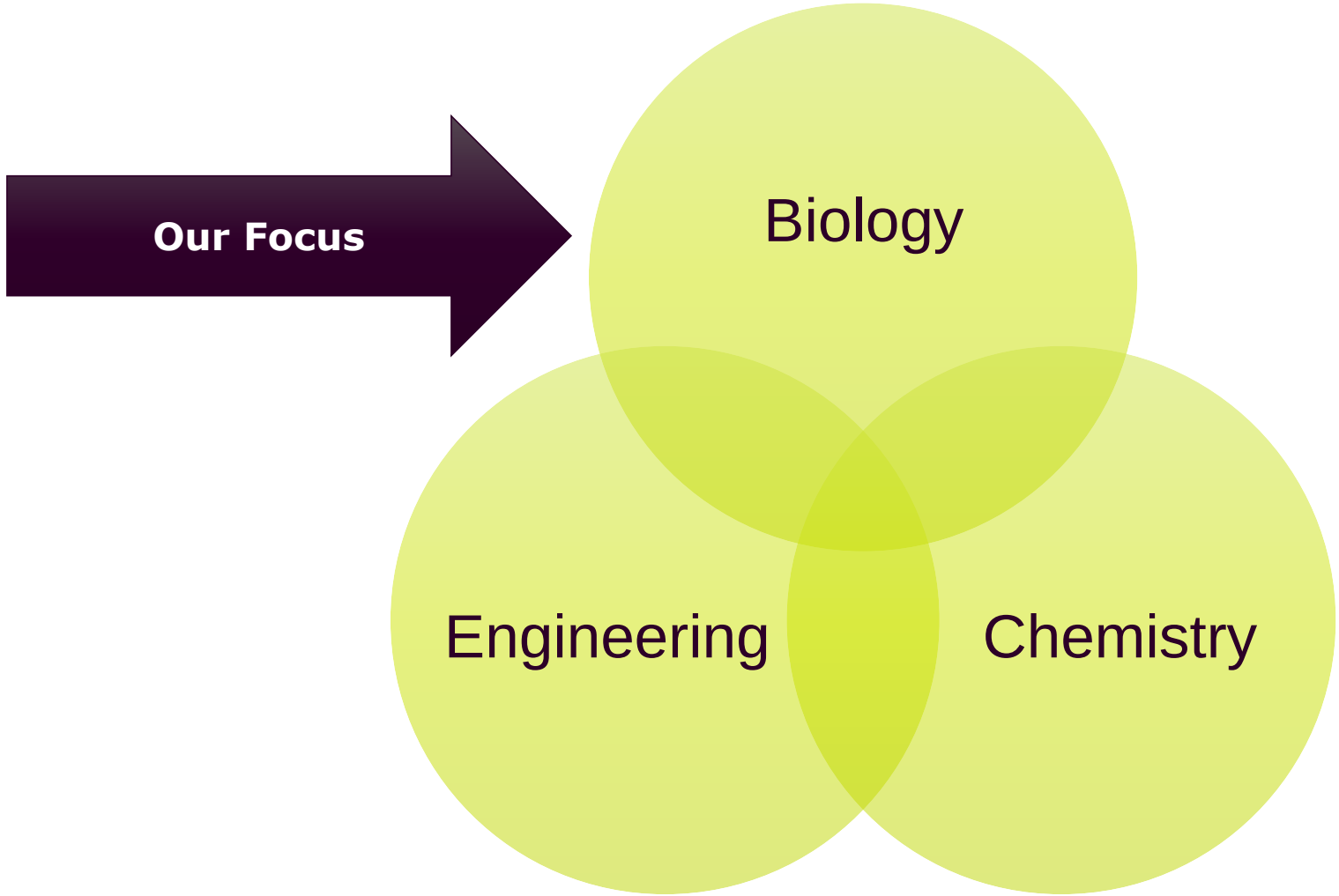
Introduction



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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.

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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-***"?

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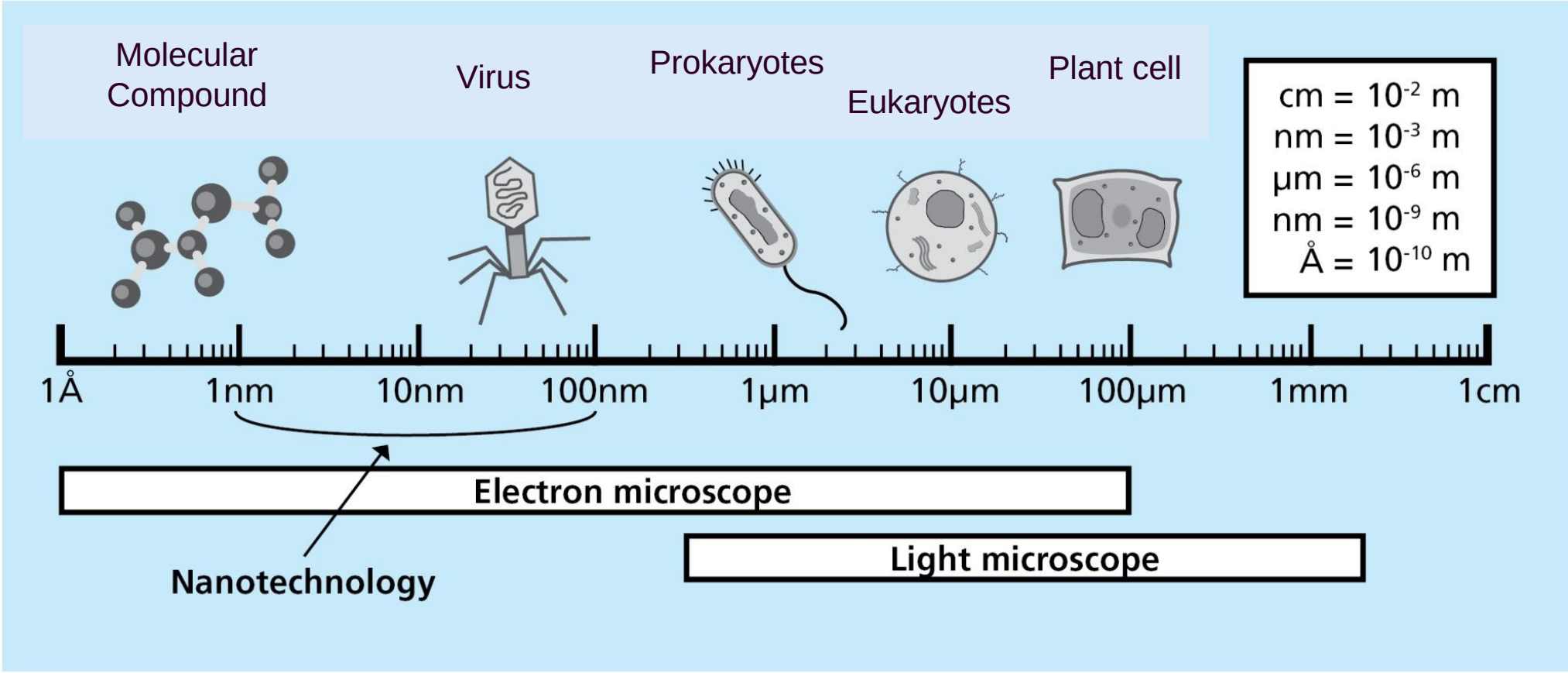
Size of Microorganism –

Typically 0.01 µm to 1 mm



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What do microorganisms need to thrive?

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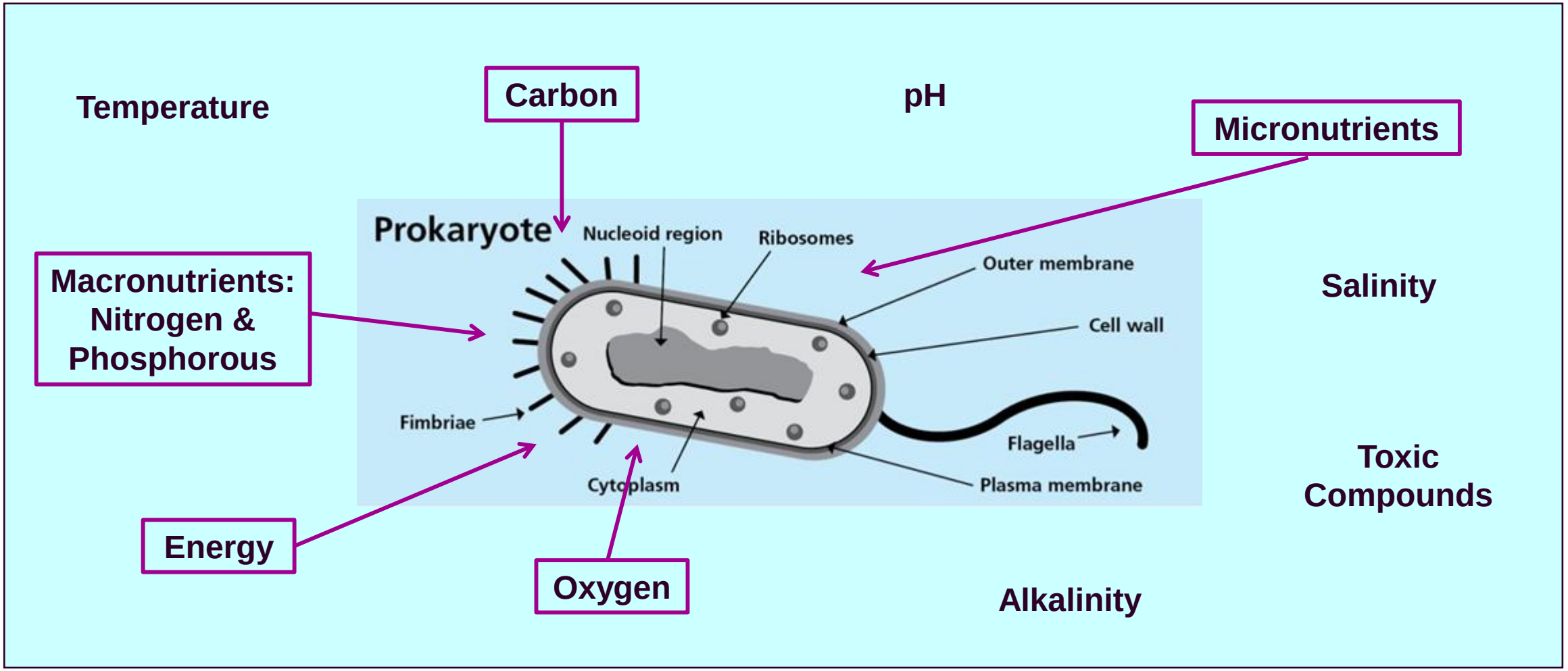
Nutrients and Environment



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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!

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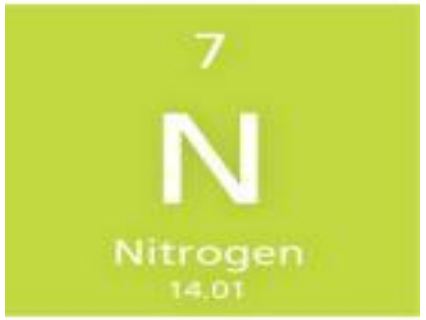
Macronutrients

100



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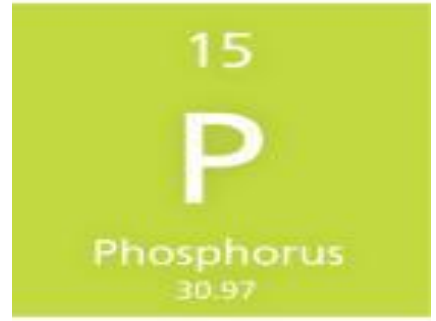

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How do you test Nitrogen and Phosphorous?

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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_2S) formation	-50 to -250
Biological phosphorus release	-100 to -250
Acid formation (fermentation)	-100 to -225
Methane production	-175 to -400

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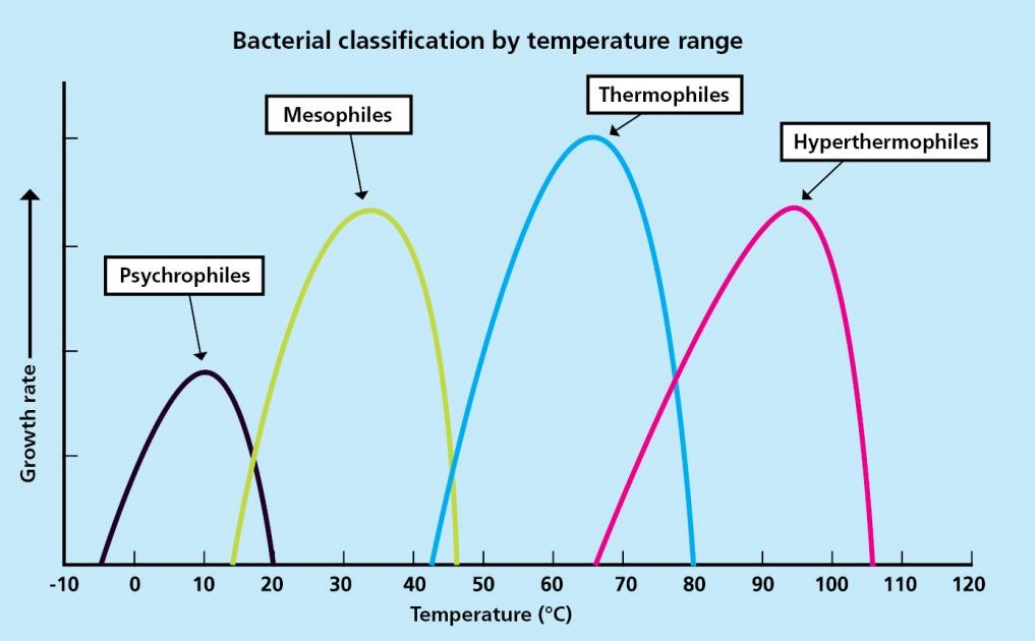
Temperature

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**

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pH

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

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.

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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 $CaCO_3$ for each ppm of NH_4 reduced
- Anaerobic processes generate acids (VFAs) during acidogenesis and acetogenesis:
  - Approx. 3/1 ratio of VFA/Alkalintiy

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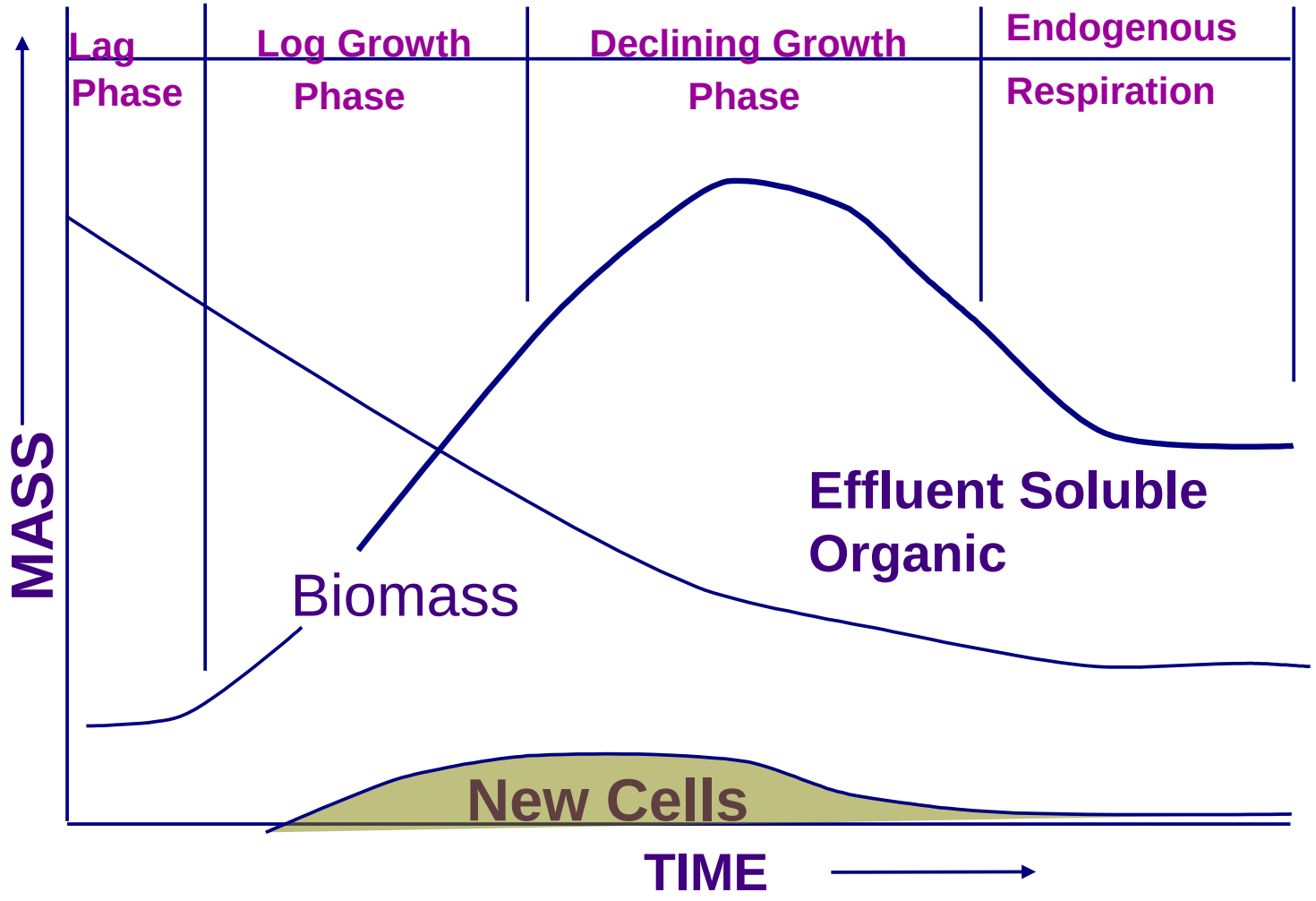
Microorganism Life Cycle

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)



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Wastewater Microscopy

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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

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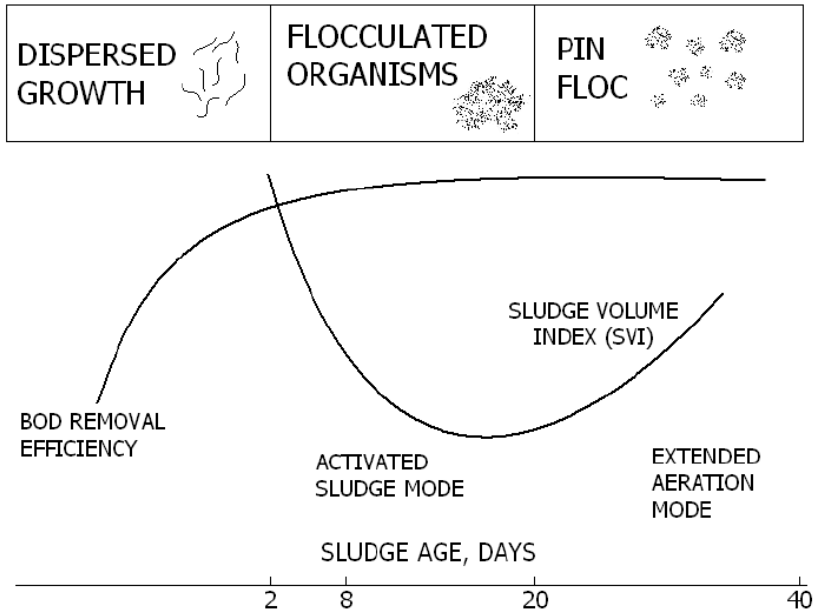
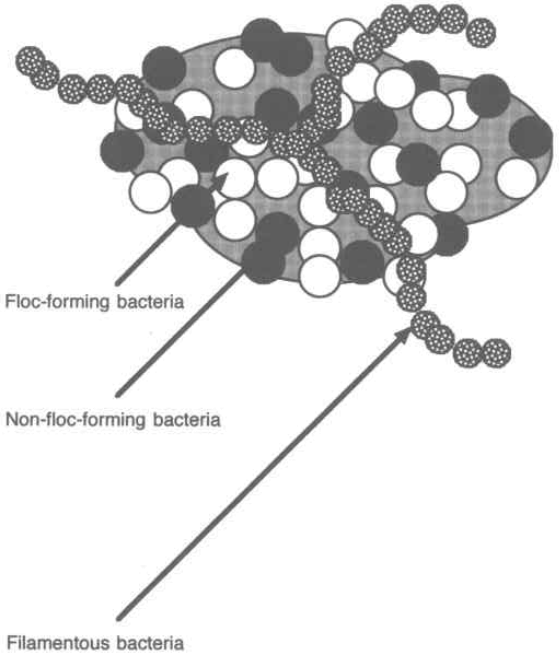
What can you do with microscopy?

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





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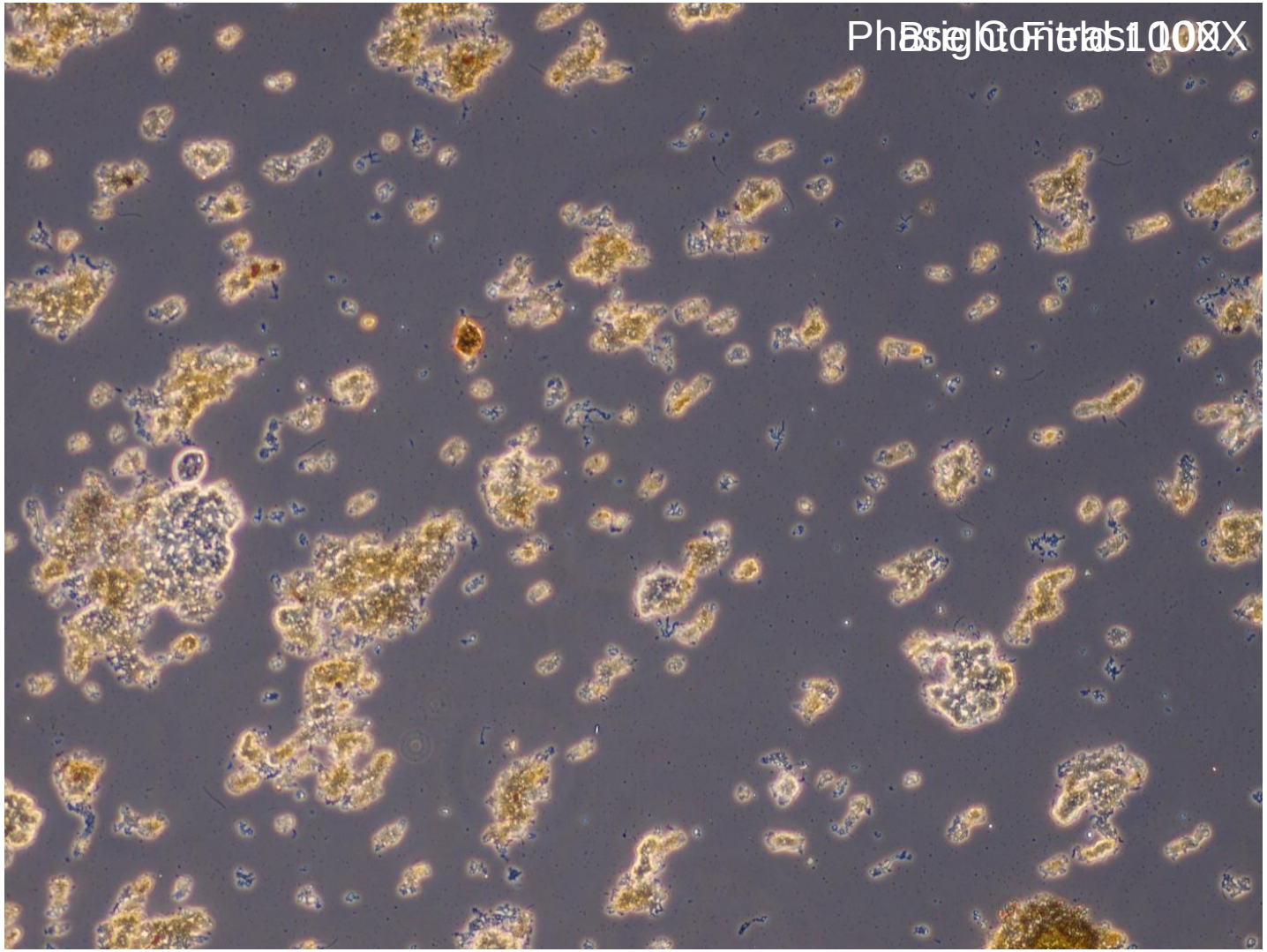
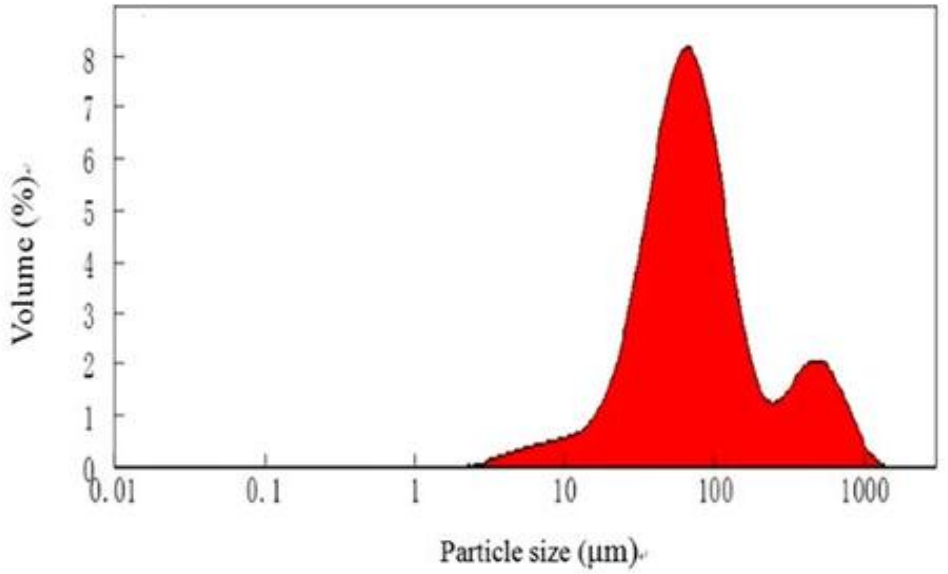
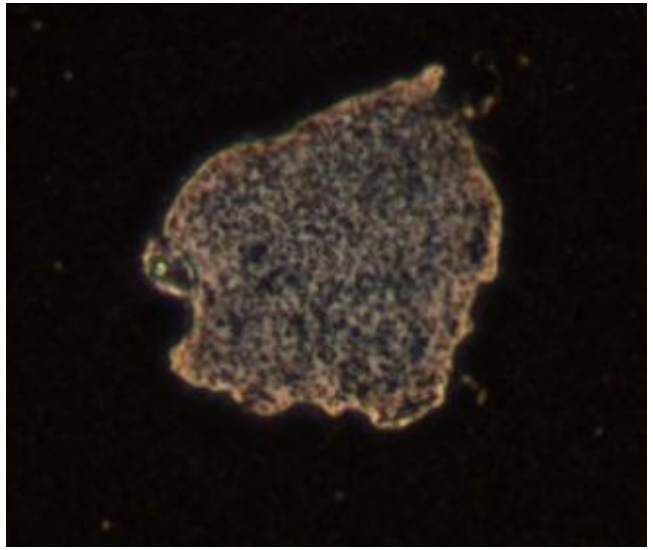
Phase contrast



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)
  - Zoogloeal growth





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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

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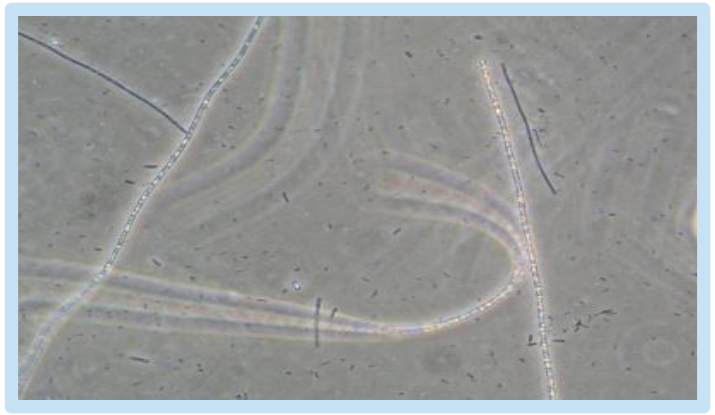
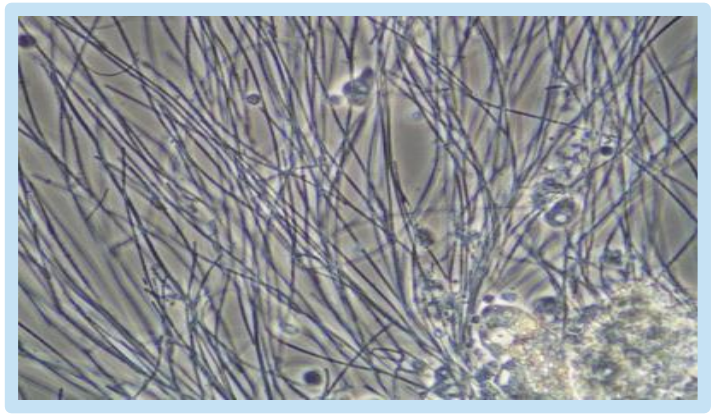
Examples of Filamentous Bacteria

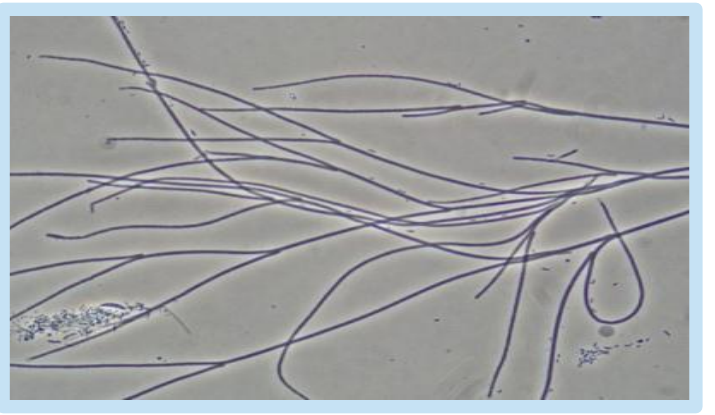
Beggiatoa



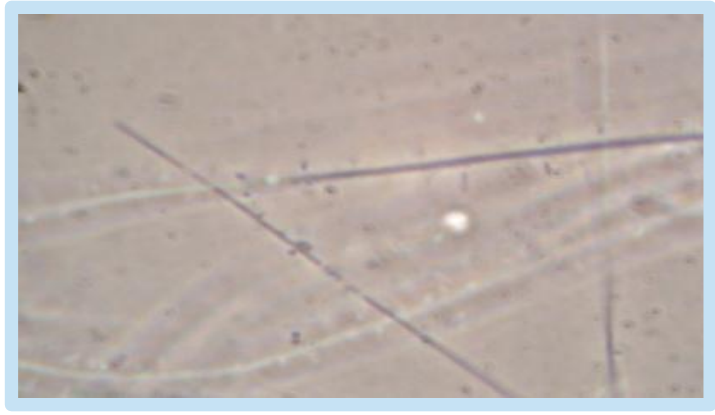
Microthrix parvicella



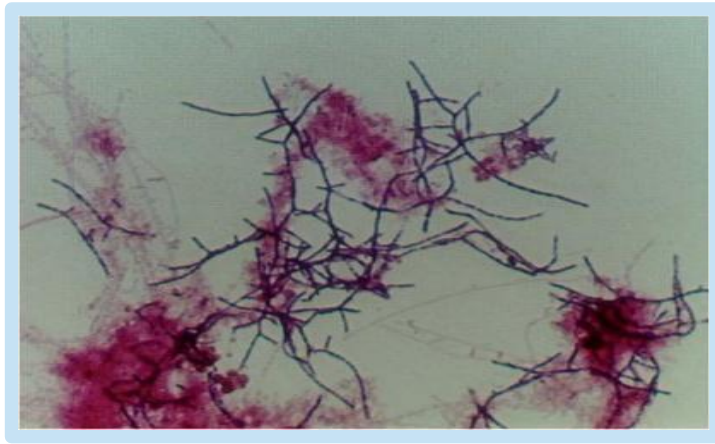
S. natans



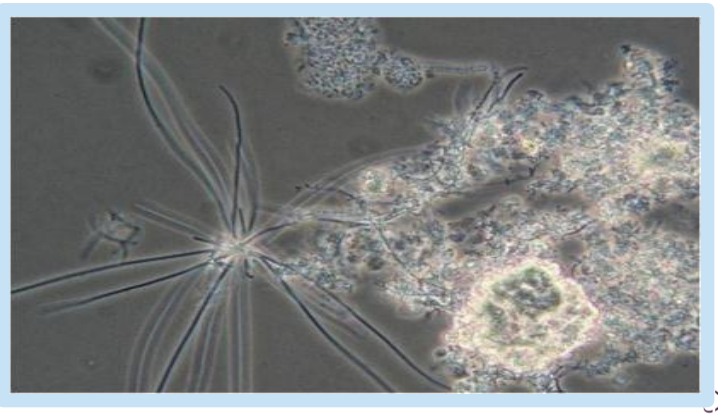
H. hydrossis



Nocardia



Thiothrix



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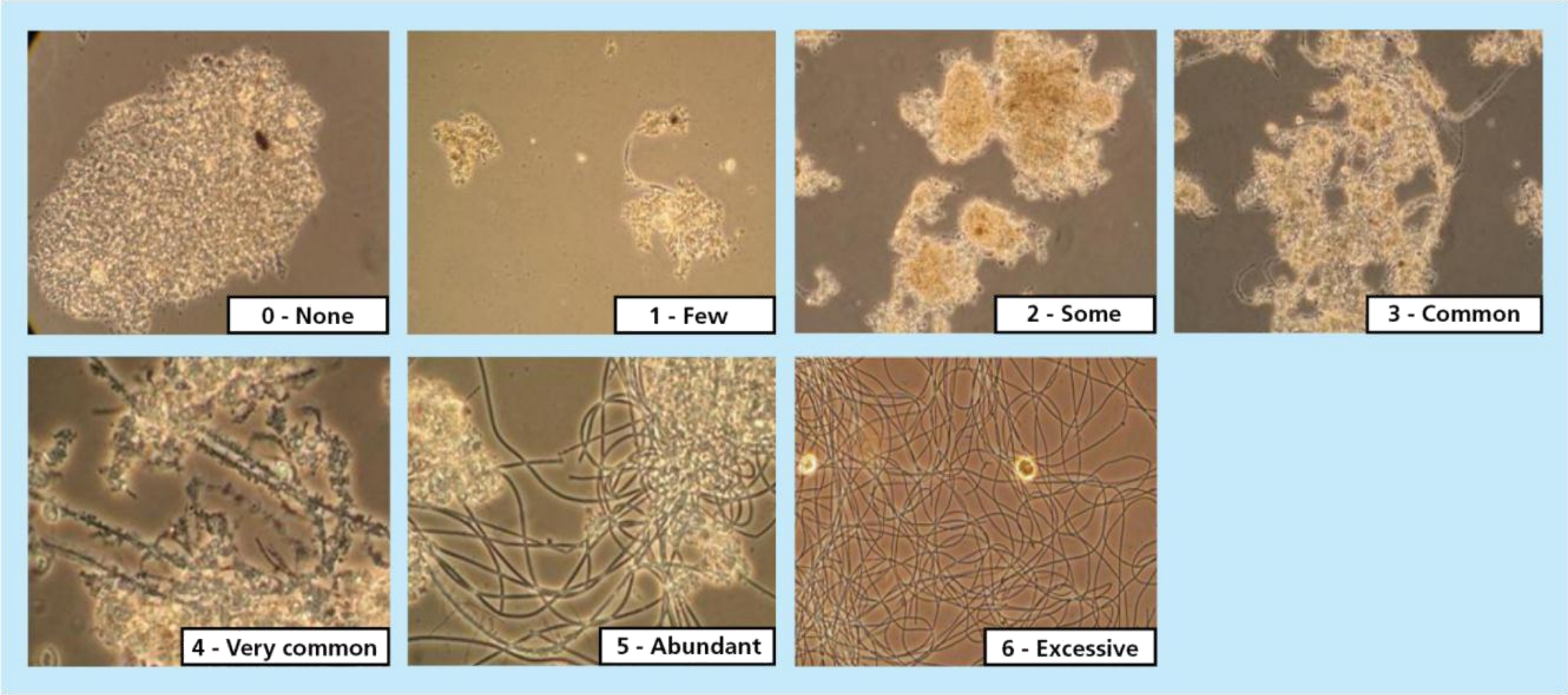
Filamentous Index



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Filaments and associated causes

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

Low Dissolved oxygen	Sphaerotilus natans, Type 1701, Haliscomenobacter hydrosis
Low F/M	Type 0041, Type 0675, Type 1851, Type 0803
Septicity	Type 021N, Thiothrix I&II, Type 0914, Type 0411, Type 0961, Type 0581, Type 0092, Nostocoida limicola I, II, and III
Oil and Grease	Nocardia sp., Microthrix parvicella, Type 1863
Nutrient Deficiency	Type 021N, Thiothrix I and II, S. natans, N. limicola III, H. hydrosis
Low pH	Algae

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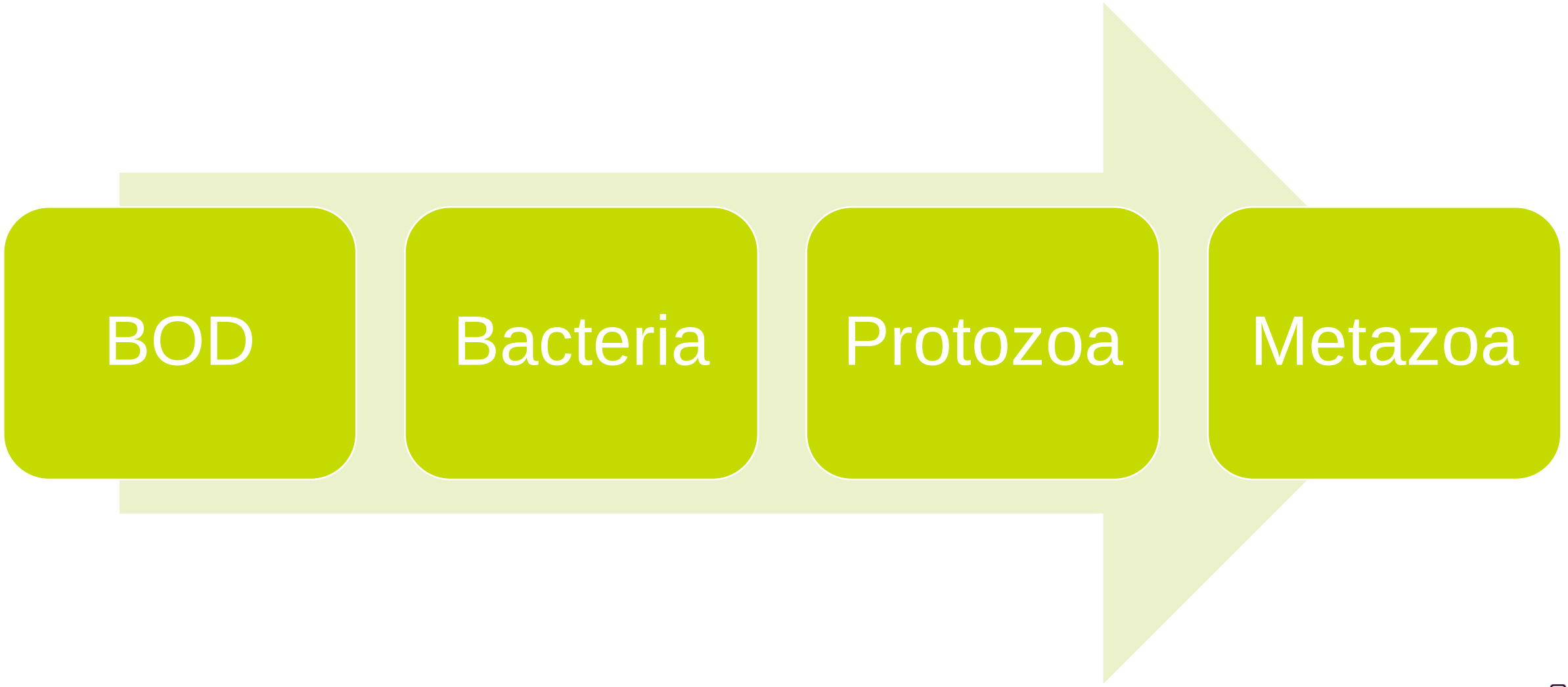
Higher Life



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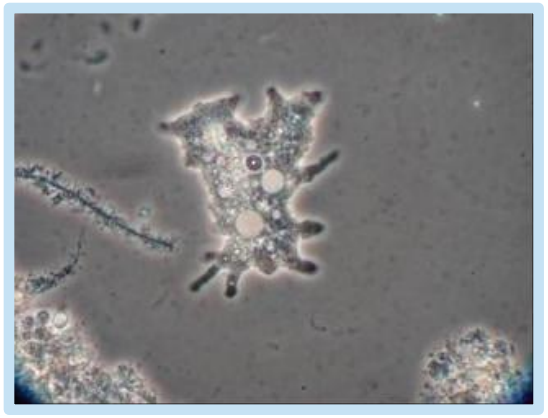
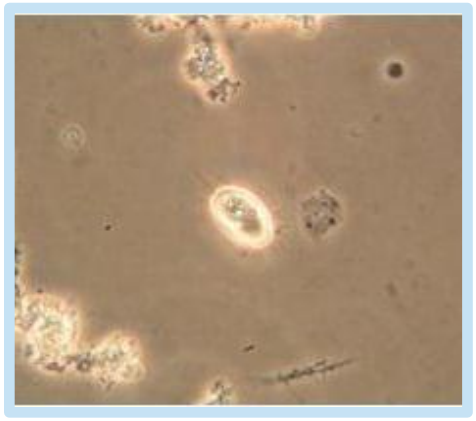
Protozoa (single celled)

Amoeba

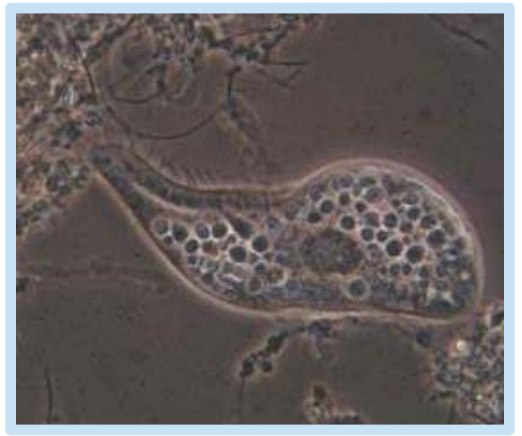


Flagellates

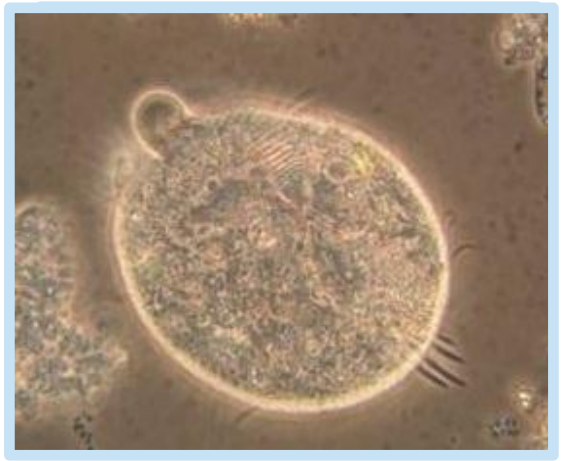
Free-Swimming Ciliates



Carnivorous Ciliates

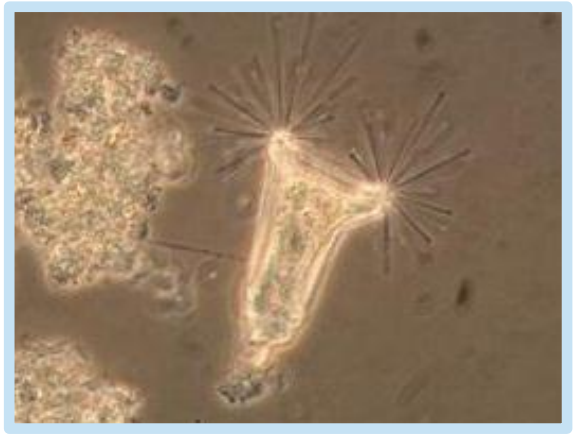


Crawling Ciliates



Stalked Ciliates

Suctoria



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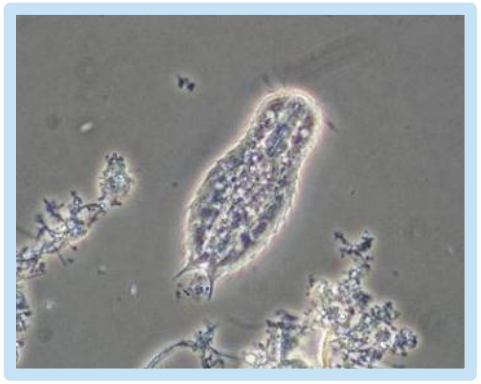
Metazoa (many celled)

Rotifers

Gastrotrichs



Aeolosoma Worms

Tardigrades

Nematodes

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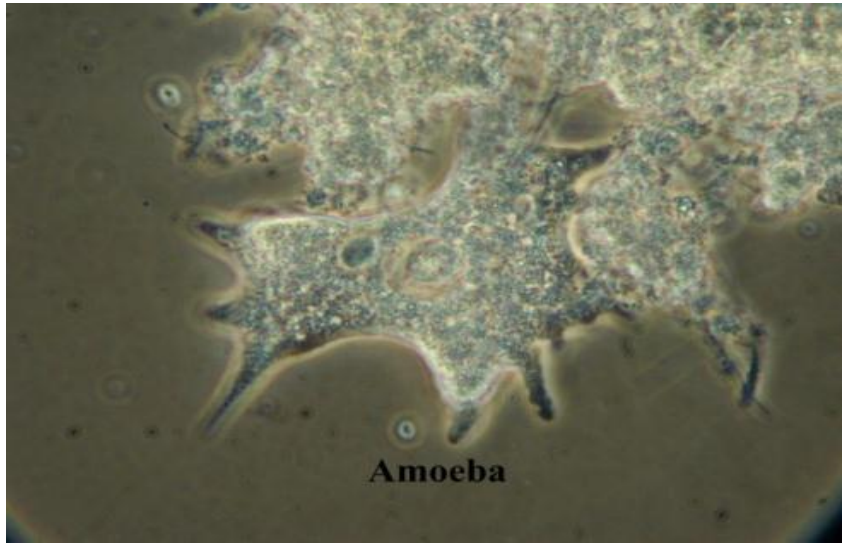
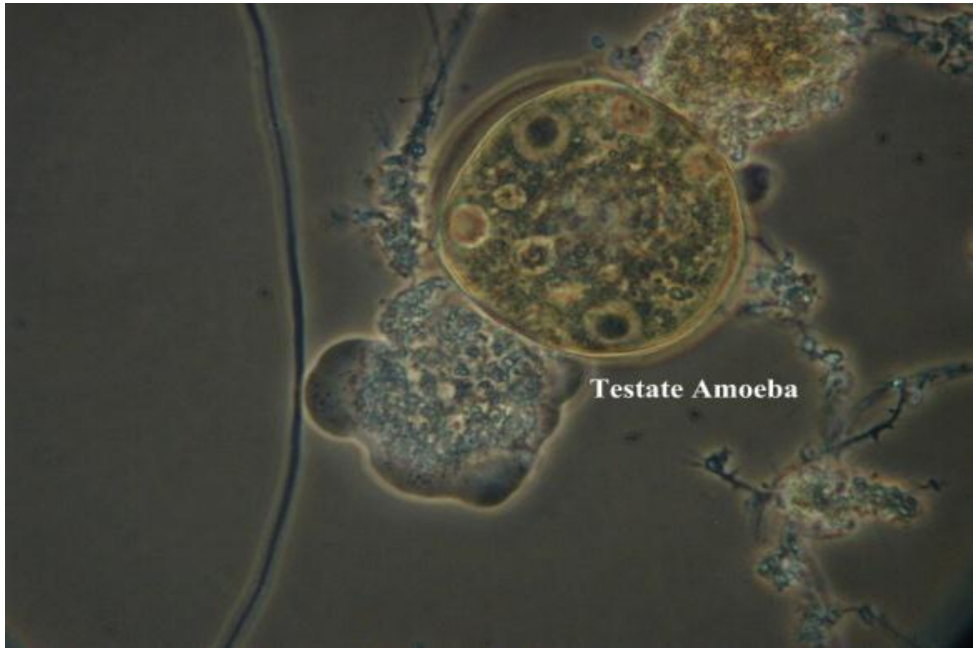
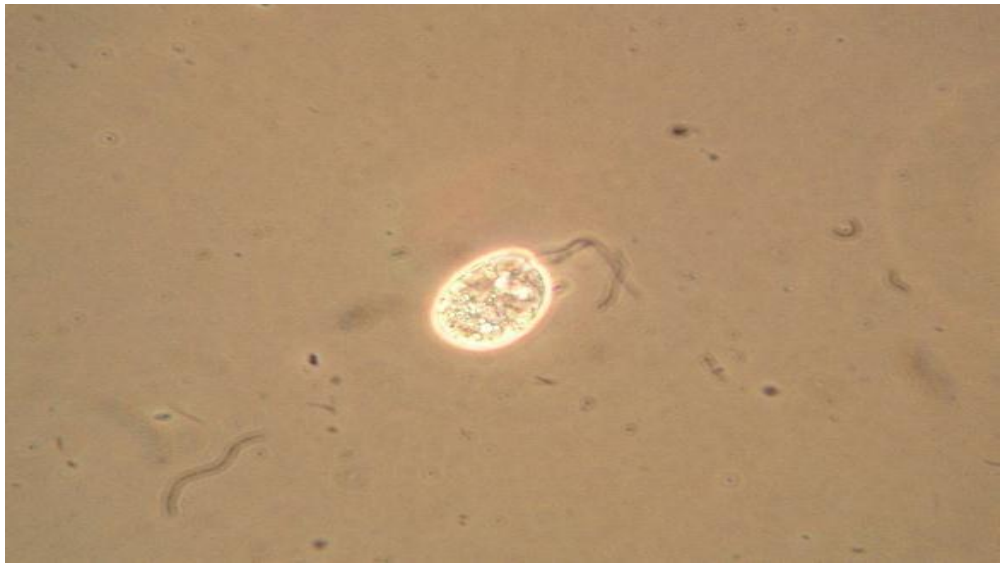
Amoeba

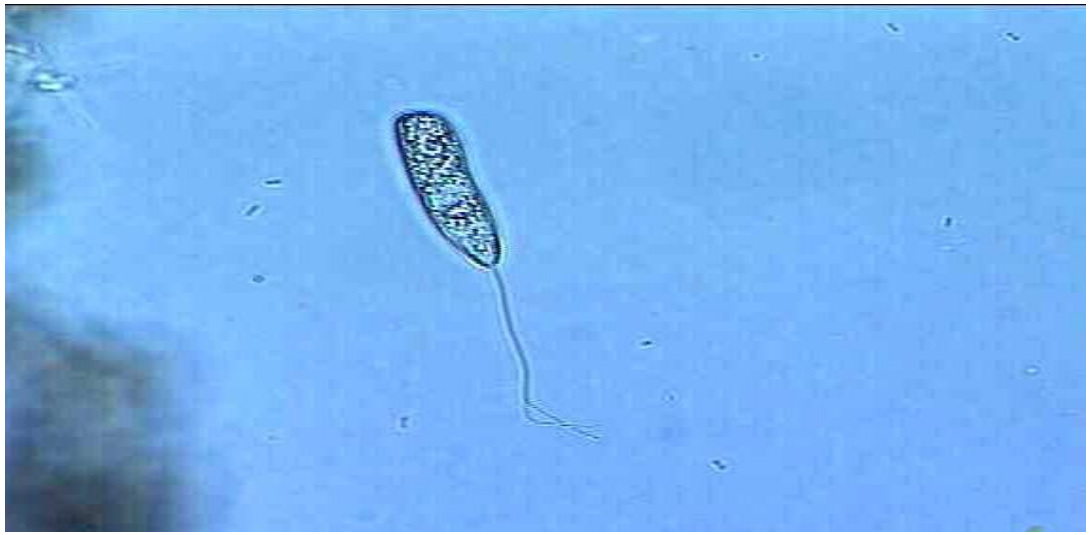


- Slowly mobile or not
- Present at start-up
- May indicate a recent upset event
- Grow well on particulate organic matter
- Can tolerate low DO



Flagellates





- Length = 10 – 25 µm
- 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 µm
- 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

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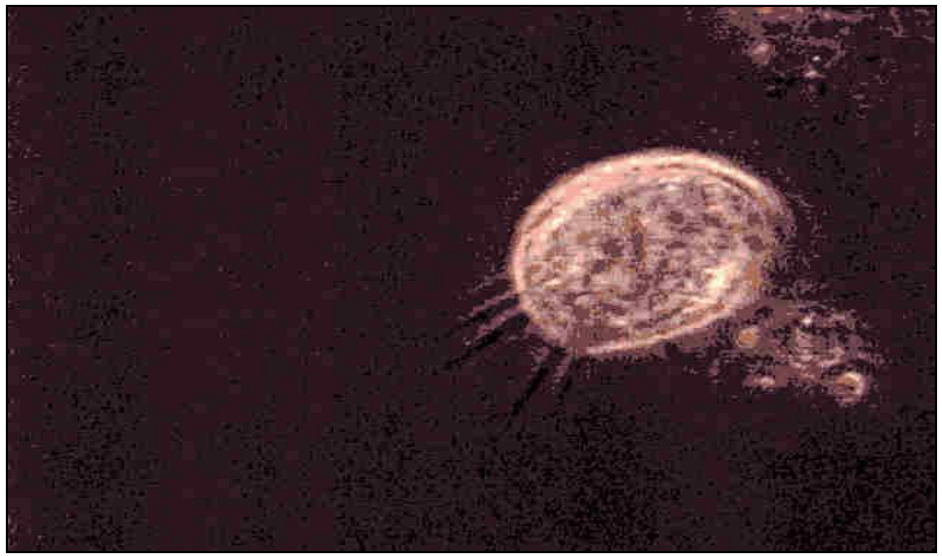
Crawling Ciliates



- Length – 25 – 60 µm
- Motile by “crawling” over floc particles
- Feed off of floc particles
- Usually occur under conditions of good floc formation
- Indicate good activated sludge operation

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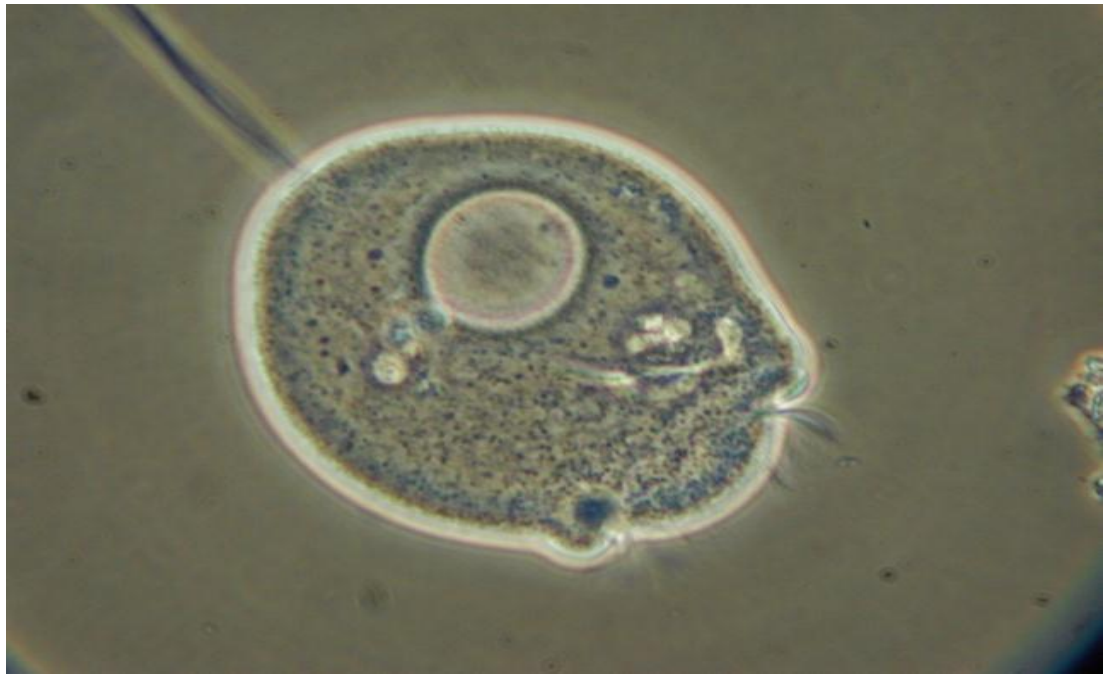
Stalked Ciliates



- Length 50 – 100 µm
- Found attached to flocs by a stalk
- Some may contract.
- Indicate stable activated sludge operation
- Colonial forms may indicate at long MCRT.

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Rotifers

- Length 100 – 500 µm
- More complex than protozoa -- multi-celled with structural zones
- Motile by a contractile “foot”
- Usually occur at higher MCRTs

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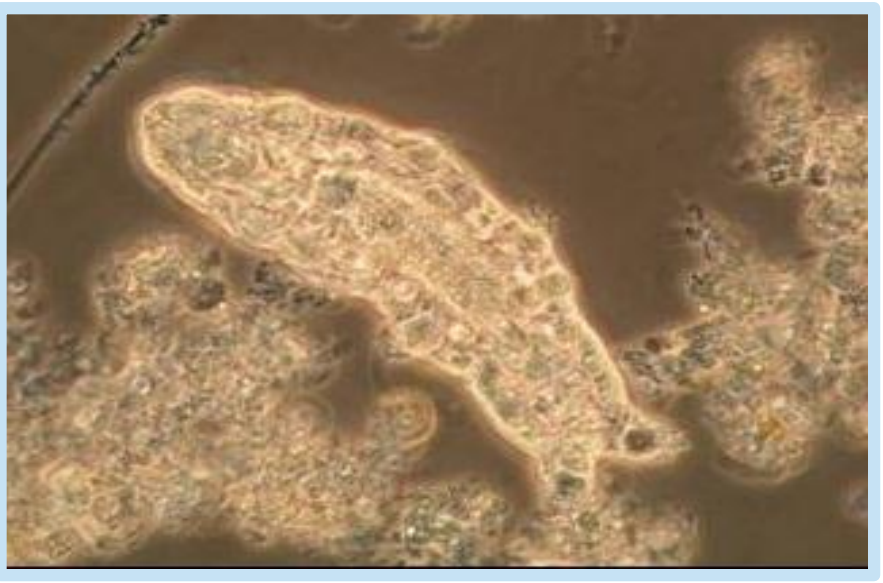
Gastrotrichs

Vago, G. 2012

- 60 µm to 3 mm long
- Cilia around the body
- 2 posterior “feet”
- Detrivore, feed on just about anything (organics, bacteria, protozoa)
- Not very indicative of plant conditions

Tardigrades (Water Bears)



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

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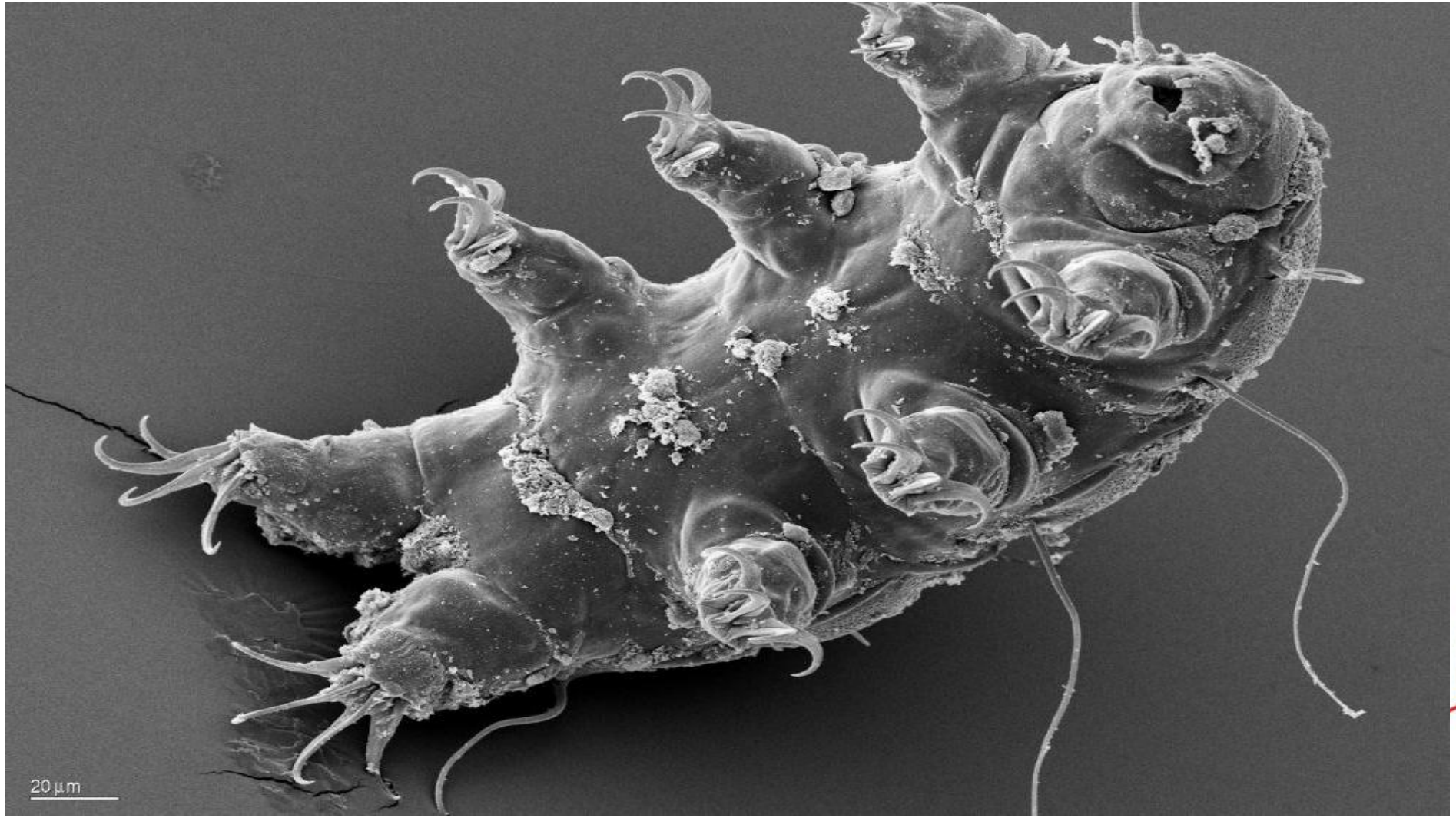

“Tolerance of Anhydrobiotic Eggs of the Tardigrade *Ramazzottius varieornatus* to Extreme Environments.” By Daiki D. Horikawa et al. Astrobiology, published online April 10, 2012.

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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**

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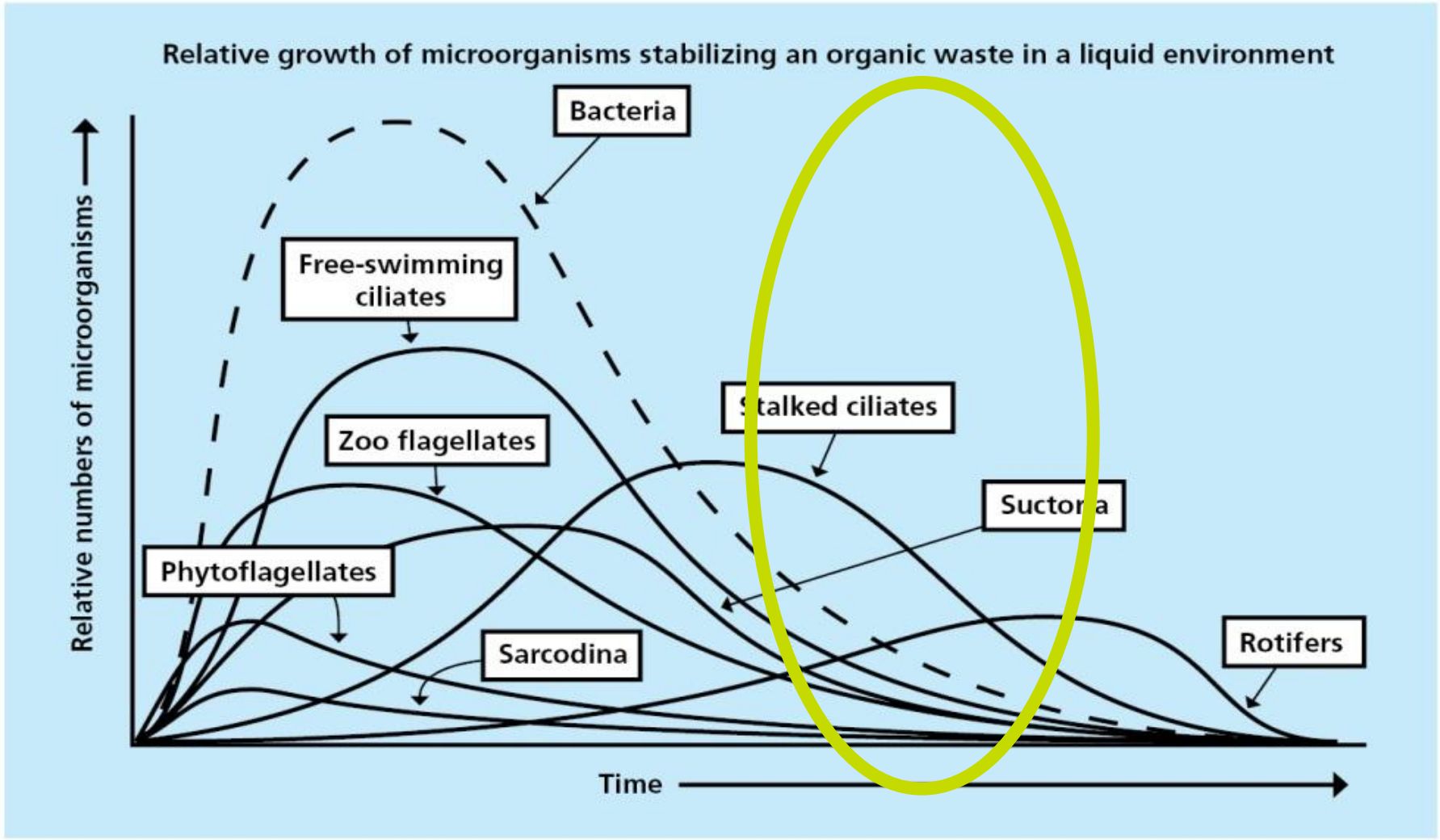
Biology of Wastewater Treatment



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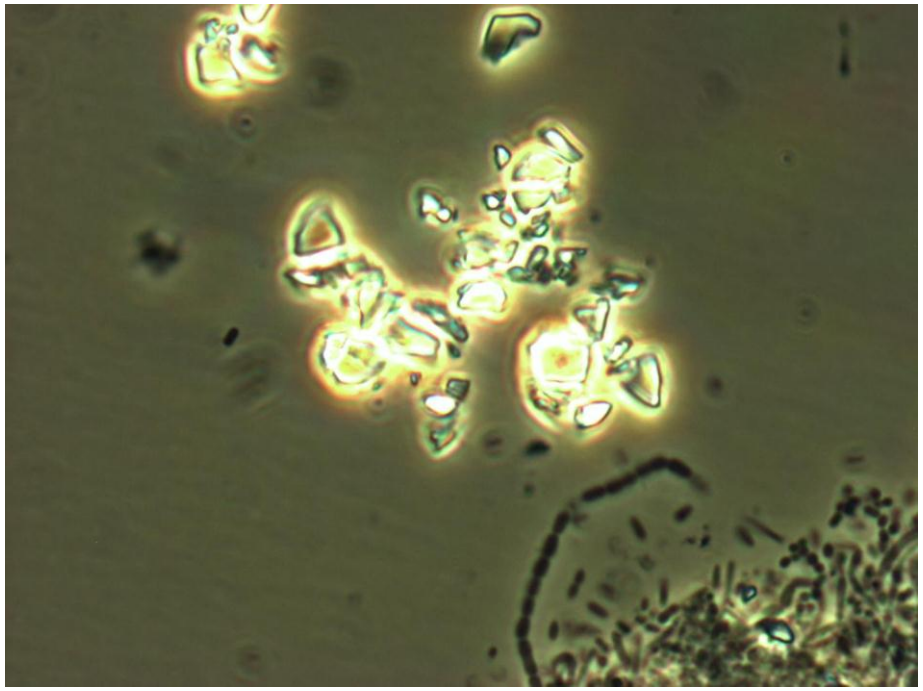
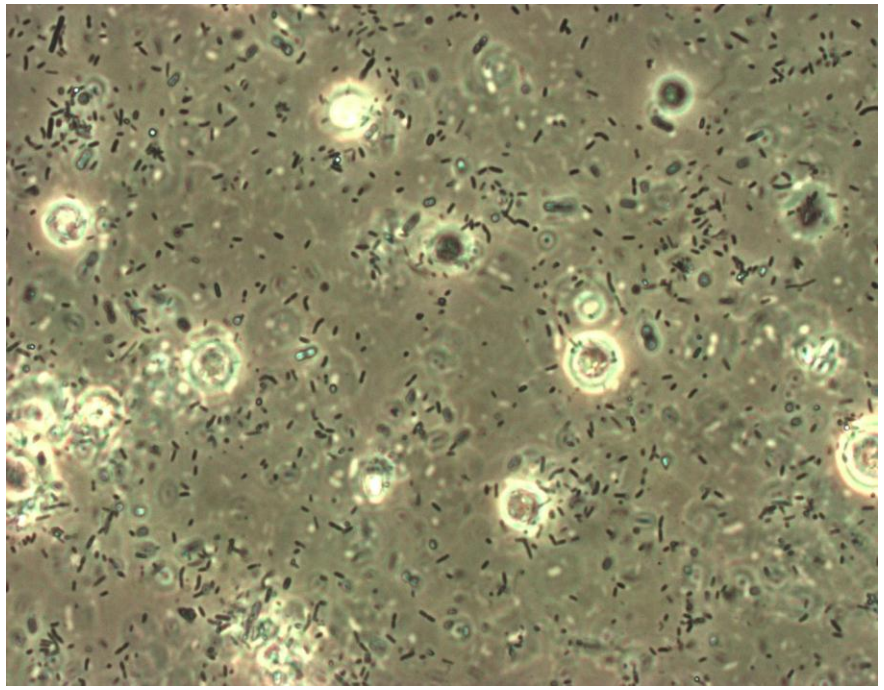
Bulk Water

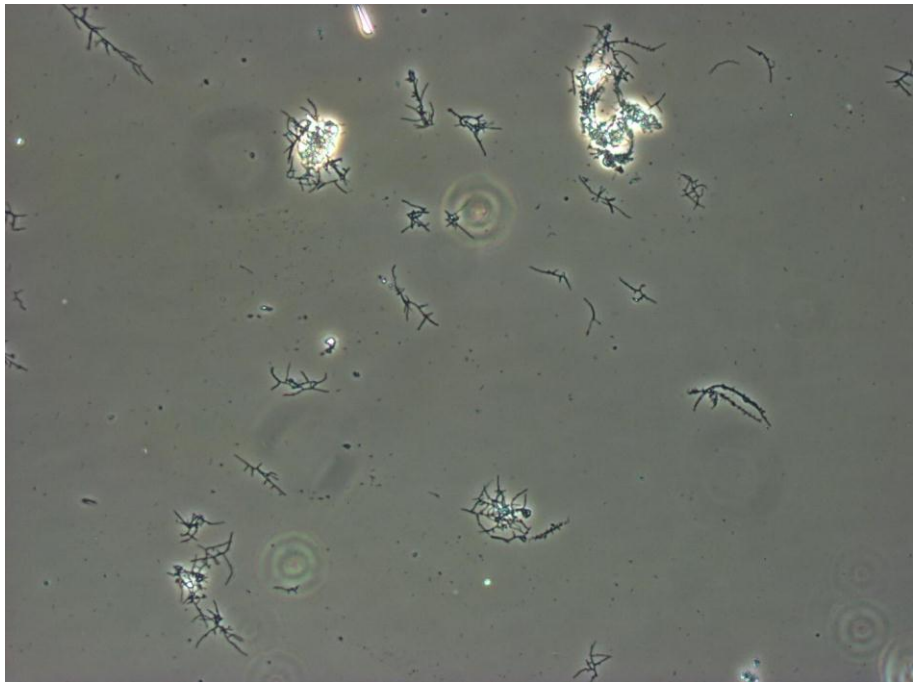
The water around the floc.

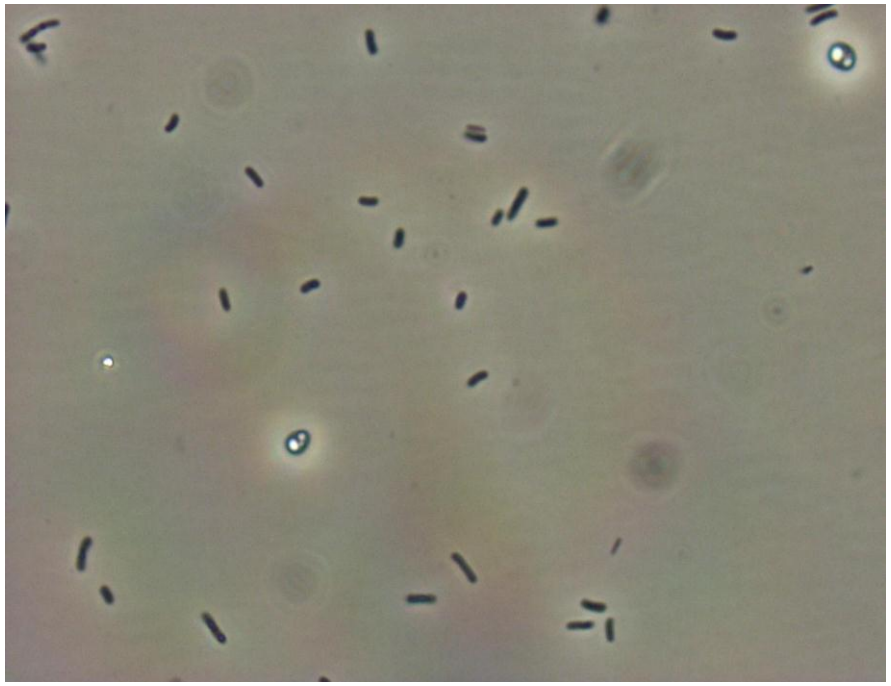
Equals secondary clarifier effluent

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









Break

Live Microscopic Examinations

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Questions

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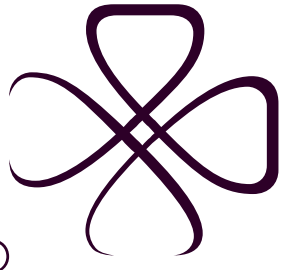

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