Probiotic® SOLUTIONS™

Microbes
Where, When, and Why

Wastewater Operator

What my mom thinks I do.

What society thinks I do.

What I tell people I do.

What non-operators think I do.

What I think I do.

What I really do.

Probiotic SOLUTIONS

Overview

- Know your Influent
- Why
- When
- Where
- Floc
- Filamentous Bacteria/Zooglea
- Foam

Overview

- Chlorination
- New Perspectives
  - PAO vs. GAO
  - DOUR
- Questions To Be Thinking About
  - What am I looking for?
  - What do I know once I have looked?
  - When should I send the sample out?

Know Your Influent

- After debris removal, influent contains:
  - 99.9% Water
  - 0.1% Solids
    - 30% of solids are suspended
    - 70% of solids are dissolved
- Chemically, influent is a 50/50 mix of inorganics and organics

Know Your Influent

- Influent fluctuates
  - Quantity Changes
    - Seasonal, I/I
    - Tourist Seasons (Rodeos, Football Games)
    - Schools/University Schedules
- Typical wastewater is 0.3 to 0.8 BOD/COD
  - BOD/COD > 0.5 easily treated by biological treatment
  - BOD/COD < 0.3 indicates toxic environment, low food, or needs biological augmentation

Why

- Knowing what microbes you have allows you to see process changes!
  - – Influent BOD, P, NH_3, FOG changes
    - Example: new restaurant districts, new industries
  - – Allows time to accommodate the changes in your system
  - – How often do you see these fluctuations
    - Diurnal, Annually, Rarely, etc.

When

- Weekly
  - Establish that baseline and collect data
    - Know what you have
  - When you have that “funny” feeling
    - Grab a sample and look, before making changes
- Before and After
  - Need to modify the system
    - Changing MLSS, aeration, cleaning schedule, etc.
  - Physical upgrades to the system

Where

- Where? Wherever you have treatment in your system:
  - Aeration
  - Anoxic
  - Foaming
  - Sludge handling
  - Before secondary settling
  - Permitted discharge point
  - Your problem areas

Where

- Consistency is key!
  - Same location at about the same time, every time
  - Always use a well-mixed, representative sample of mixed liquor or lagoon water
  - Always use the same volume or number of drops on the slide.
  - Consistent errors are easier to fix!

Where

- Additionally
  - Chart treatment system parameters during the good and bad times
    - Everyone reports changes! No Lone Rangers!
  - Measure the parameters routinely and consistently.
    - If an outside party collects your samples, monitor them a couple times a year to see how and where they sample.
  - Tracking helps identify how parameter changes are impacting your system and your effluent.

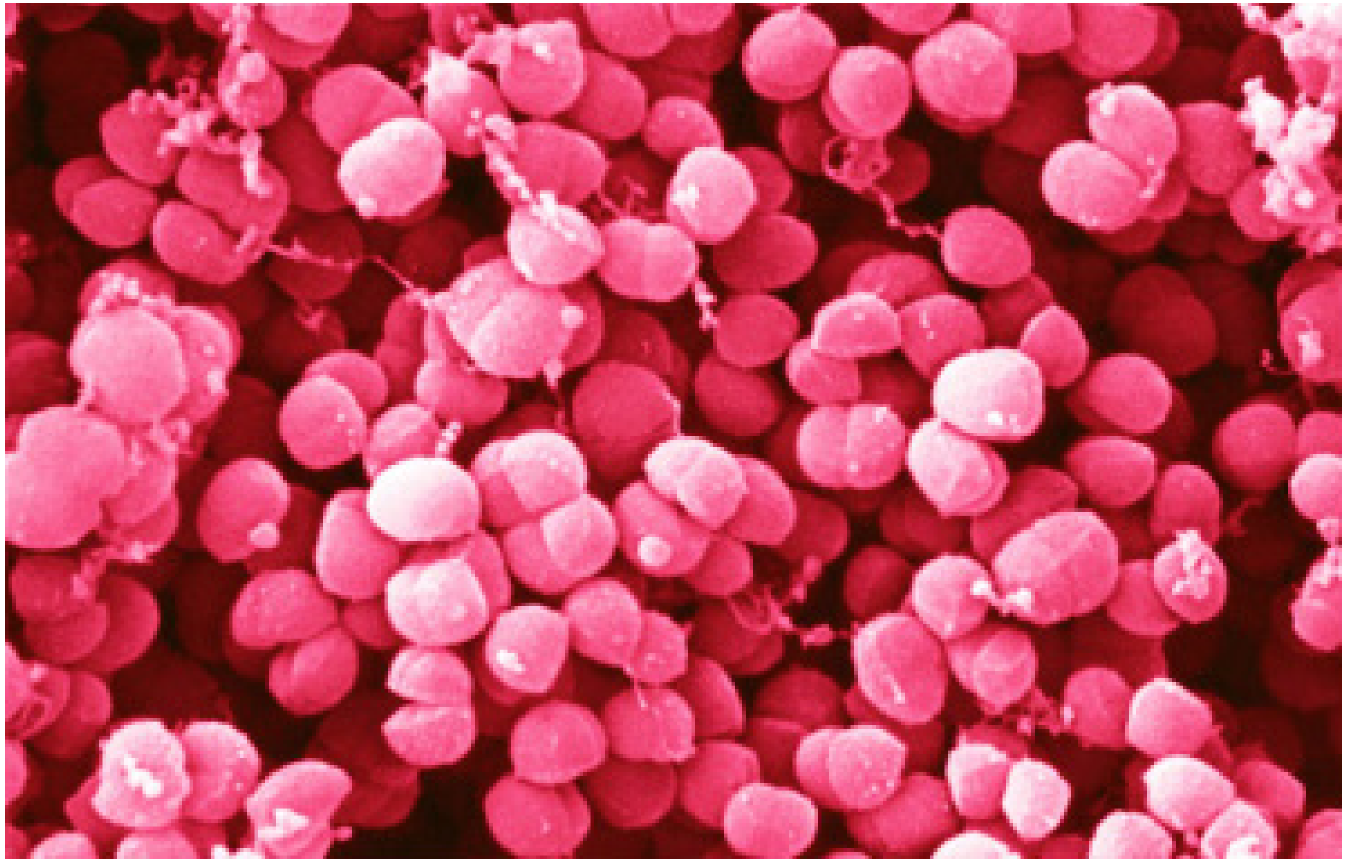
What Am I Looking For

What Am I Looking For

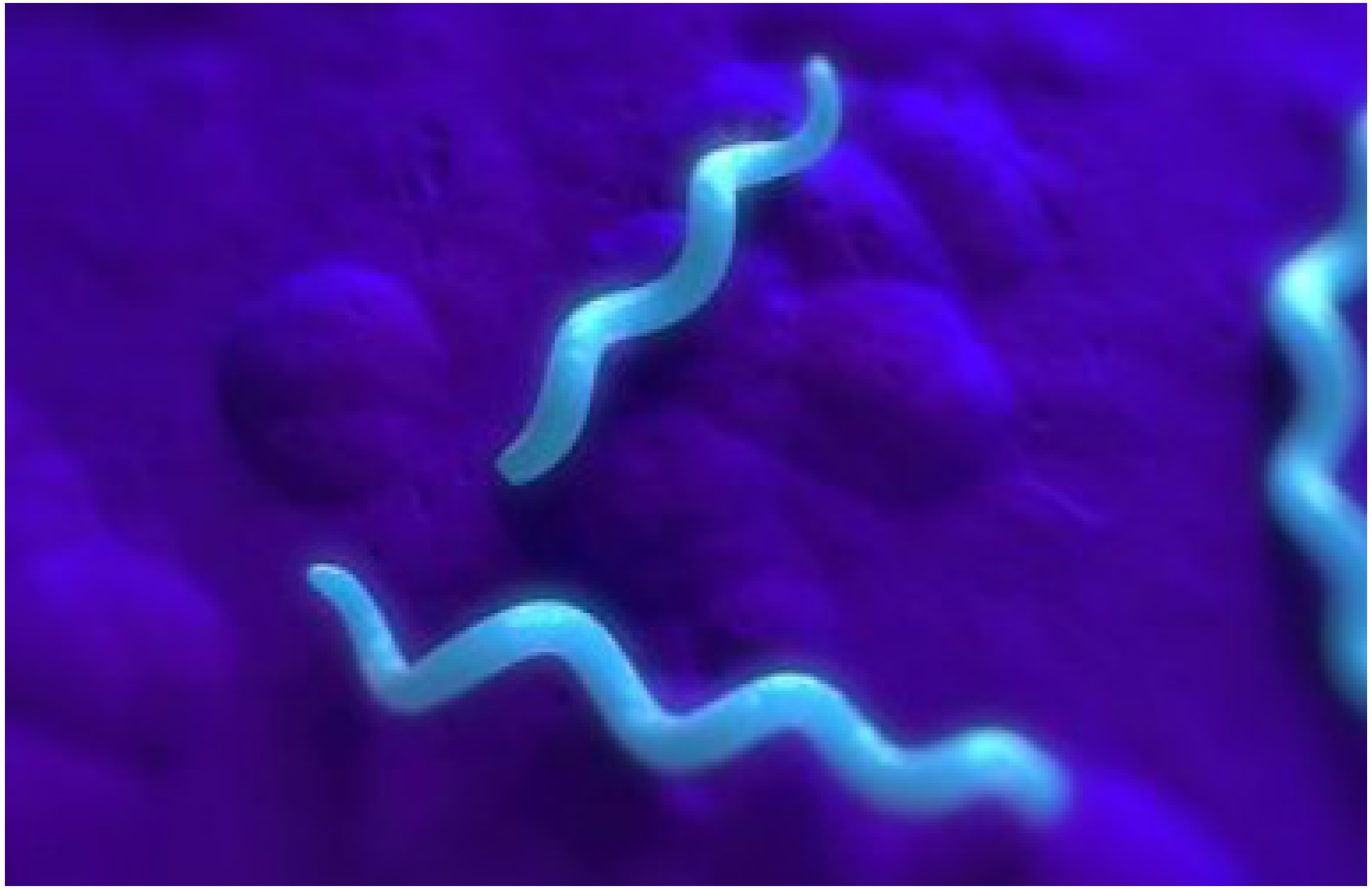
- Your Favorite Trio
  - Bacteria: they are the rank and file of the work force, about 95% of life in wastewater.
  - Protozoa and Metazoa: assist, make up 5%
- Bacteria are single-celled microorganisms that come in three basic shapes:
  - Bacillus: rod shaped, square, or rectangular,
  - Coccus: round or oval-shaped
  - Spirillum: spiral or corkscrew-shaped
- Bacteria can only consume <u>soluble</u> organic material.

Bacillus

Coccus



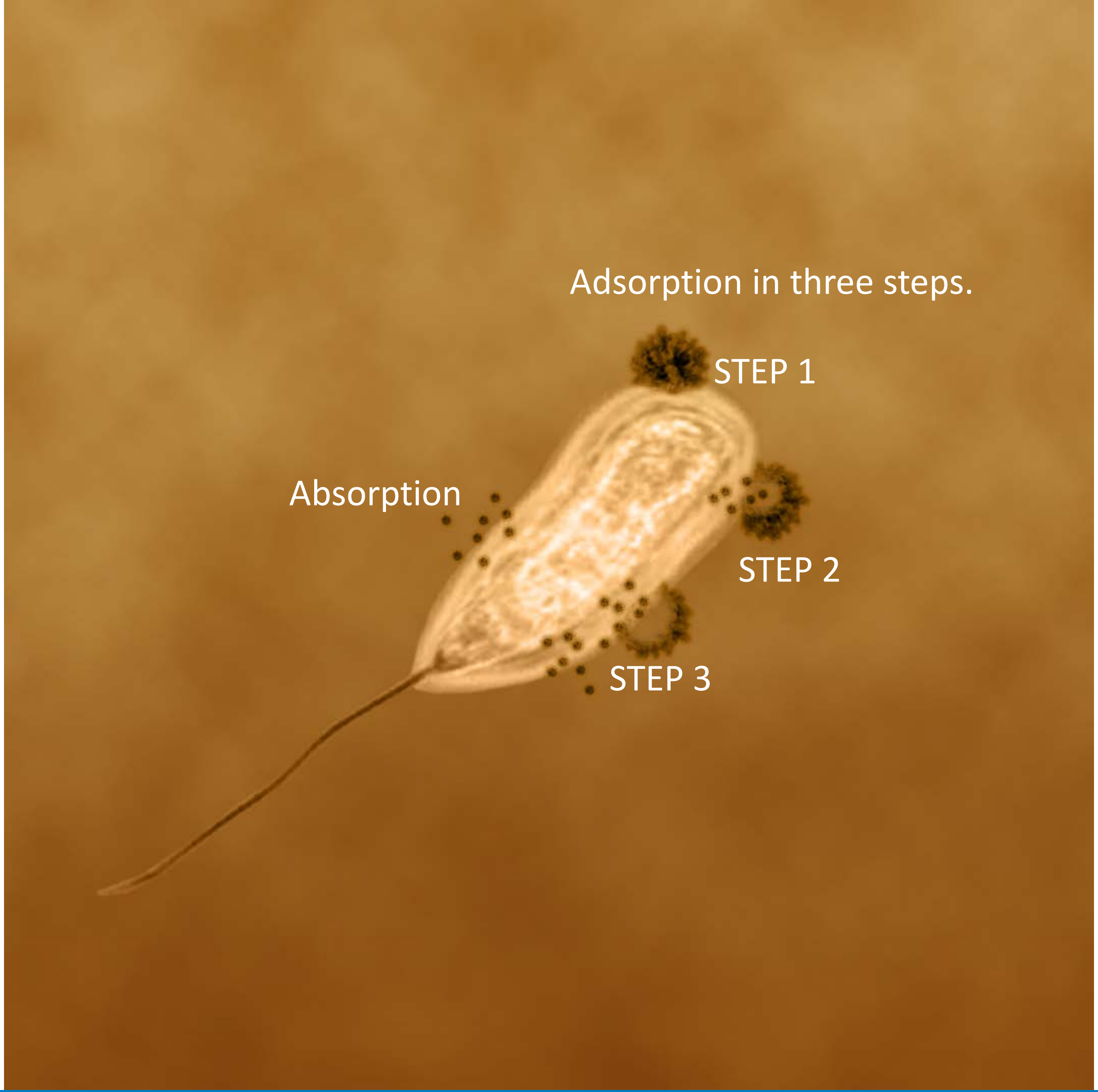
Spirillum



Bacteria

- Solid particles of “food” must be eaten by a two-step process.
  - Absorption
    - Small soluble units of food can now pass through the bacteria’s cell wall.
  - Adsorption
    - Food particles and bacteria that are too big to pass through the cell membrane that stick to the cell
    - The bacteria then secrete enzymes, which dissolve food particles into very small units, making food available for absorption

Bacteria: Ad sorption & Ab sorption

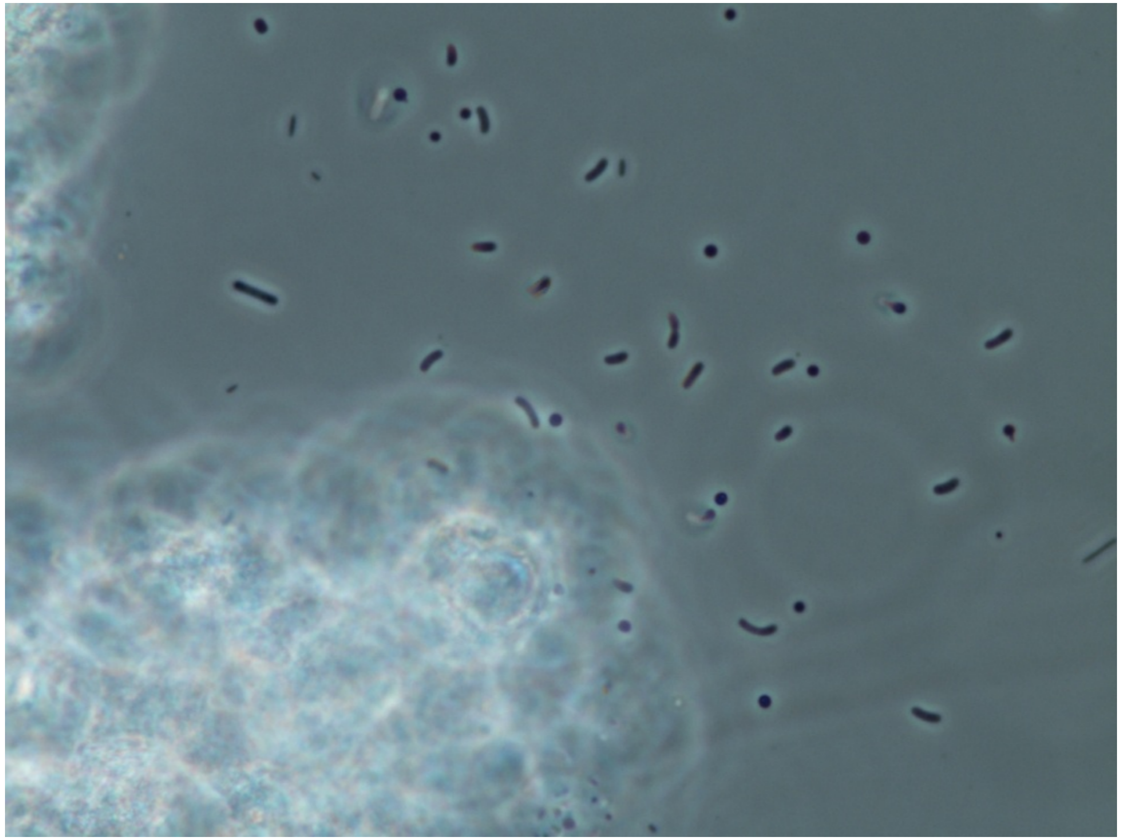


Probiotic SOLUTIONS

Bacteria

- Dispersed growth is a population of suspended, growing, non-flocculated bacteria, algae or fungi (most is bacteria)
  - If the growth rate is too fast
  - Can result in very turbid effluent
  - Often occurs after a toxicity or hydraulic washout event when the activated sludge biomass is low and high F/M conditions exist
    - Increasing MLSS can help resolve hydraulic washout issues

Dispersed Growth



Protozoa

- **Protozoa:**
  - They are single-celled organisms.
  - A single cell performs all functions; there is no division of labor.
  - They have a cellular grade of organization
  - Examples: amoeba, paramecium, flagellates, suctorians, and ciliates
  - Main defense is encystment or creation of a hard shell
    - Example: Shelled amoebas

Metazoa

- **Metazoa:**
  - They are multi-cellular organisms.
  - They show division labor as different cells or organs that perform different functions.
  - They have cellular tissue, organs, and system grade of organization.
    - Examples: rotifers, nematodes, tardigrades (water bears)

Factors That Impact Microbial Growth

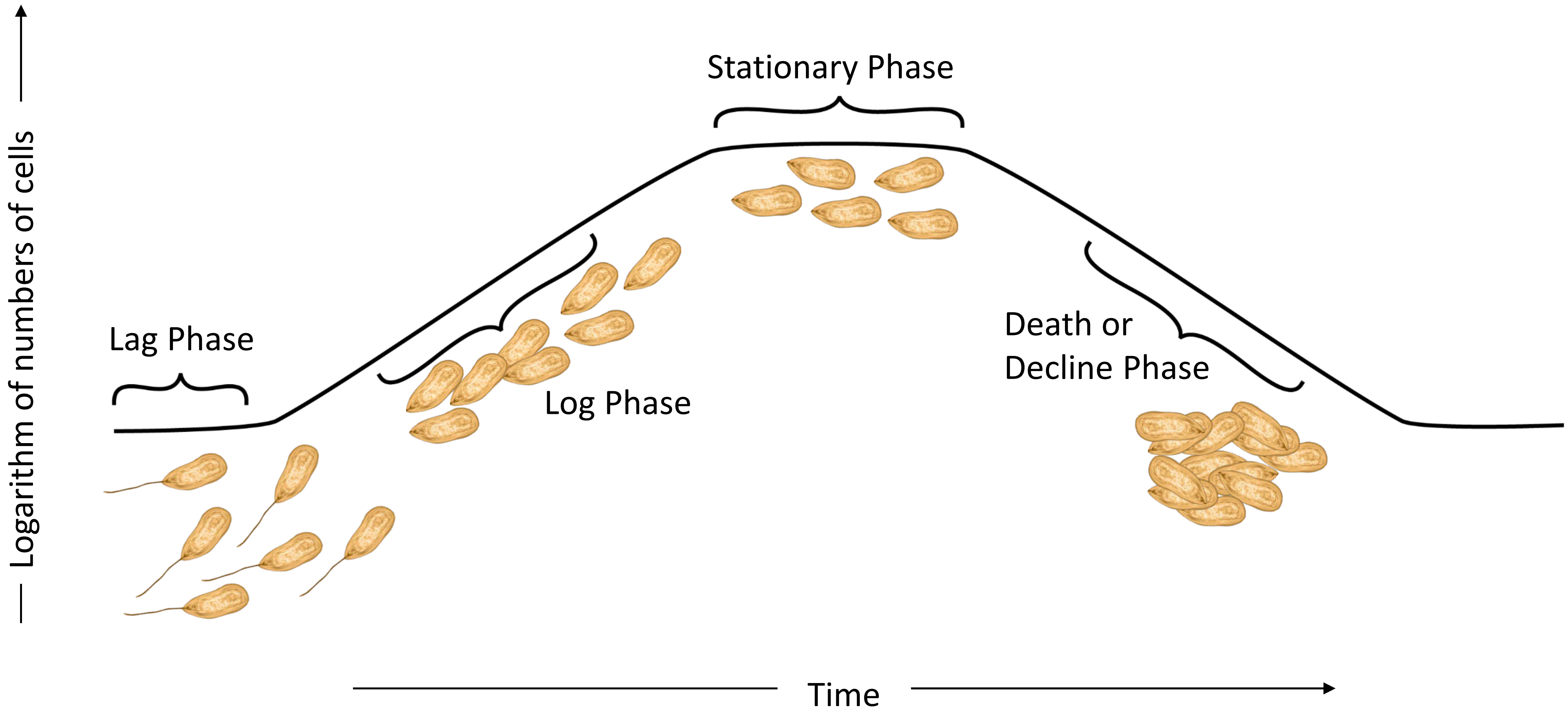
- ***Mixing***: microbial contact with food
- ***Temperature***
- ***pH***
- ***Osmotic pressure***
- ***Chemical/Nutrient requirements***
  - Carbon
  - Nitrogen, sulfur, and phosphorous
  - Trace elements/nutrients
  - Oxygen
- ***Toxicity***

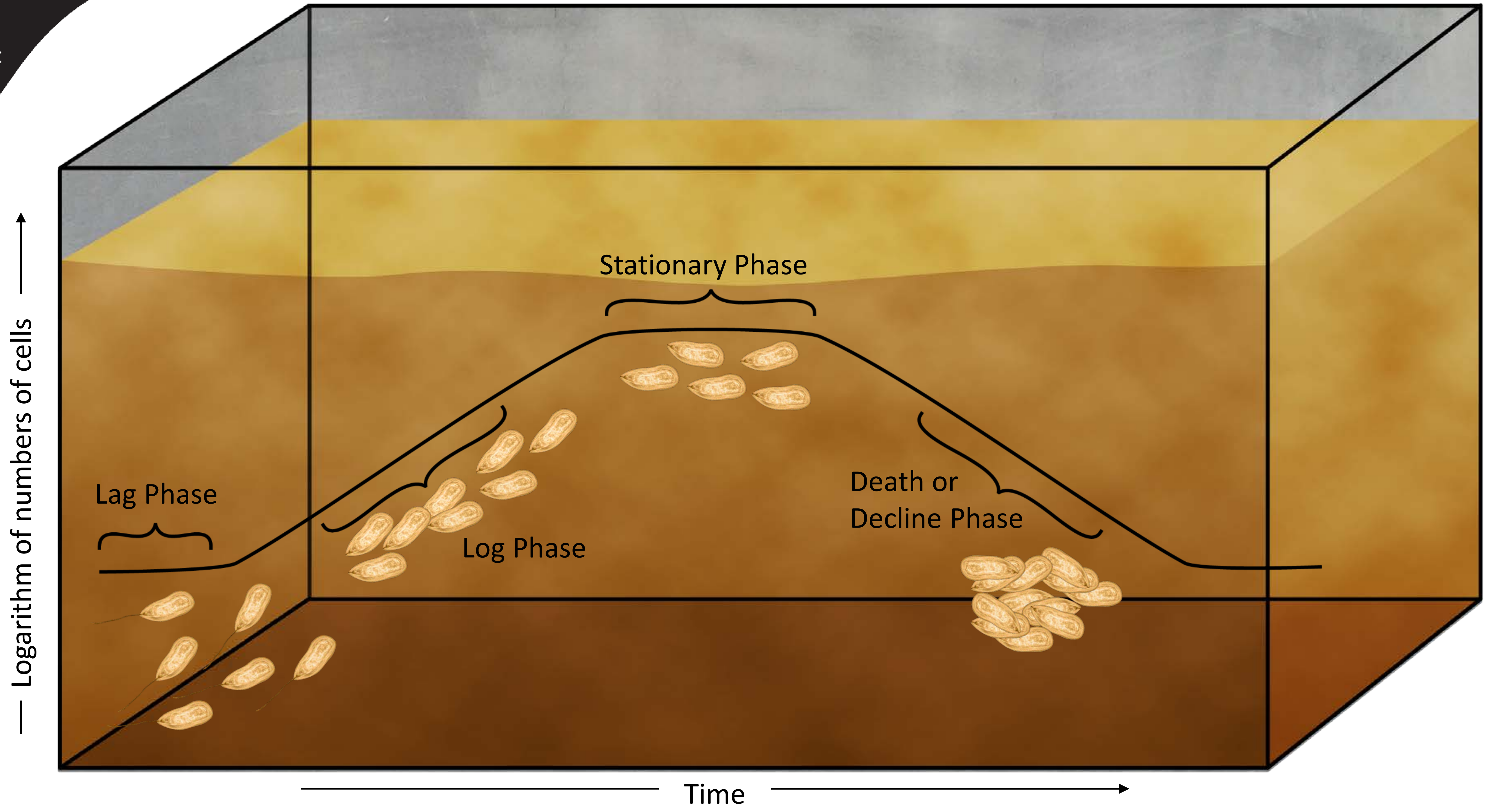
Factors That Impact Microbial Growth

- BOD:Nitrogen:Phosphorus (B:N:P) is 100:10:1.
- When conditions are unfavorable, protozoa and metazoa that form shells will dominate in the system.

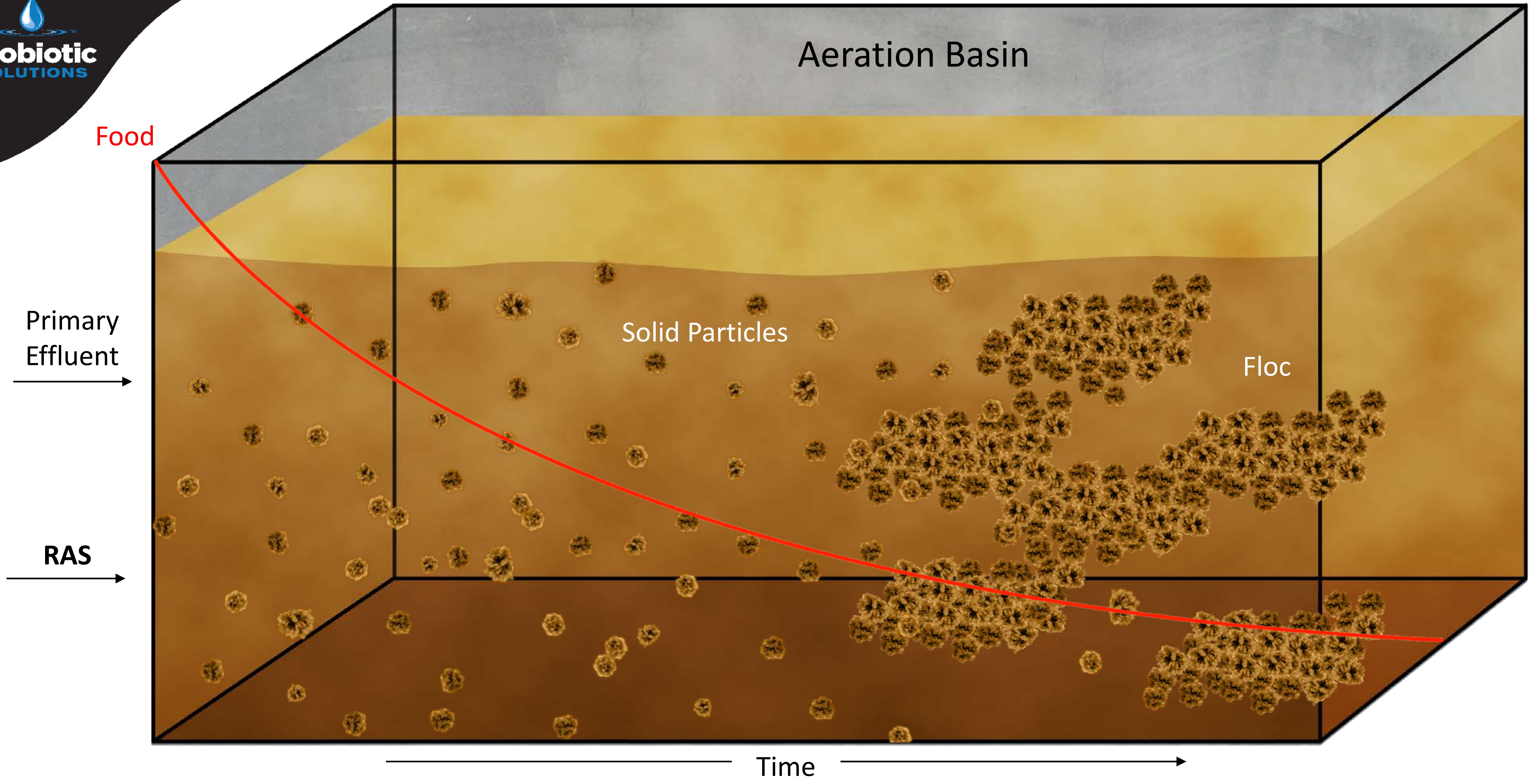
Growth Curve

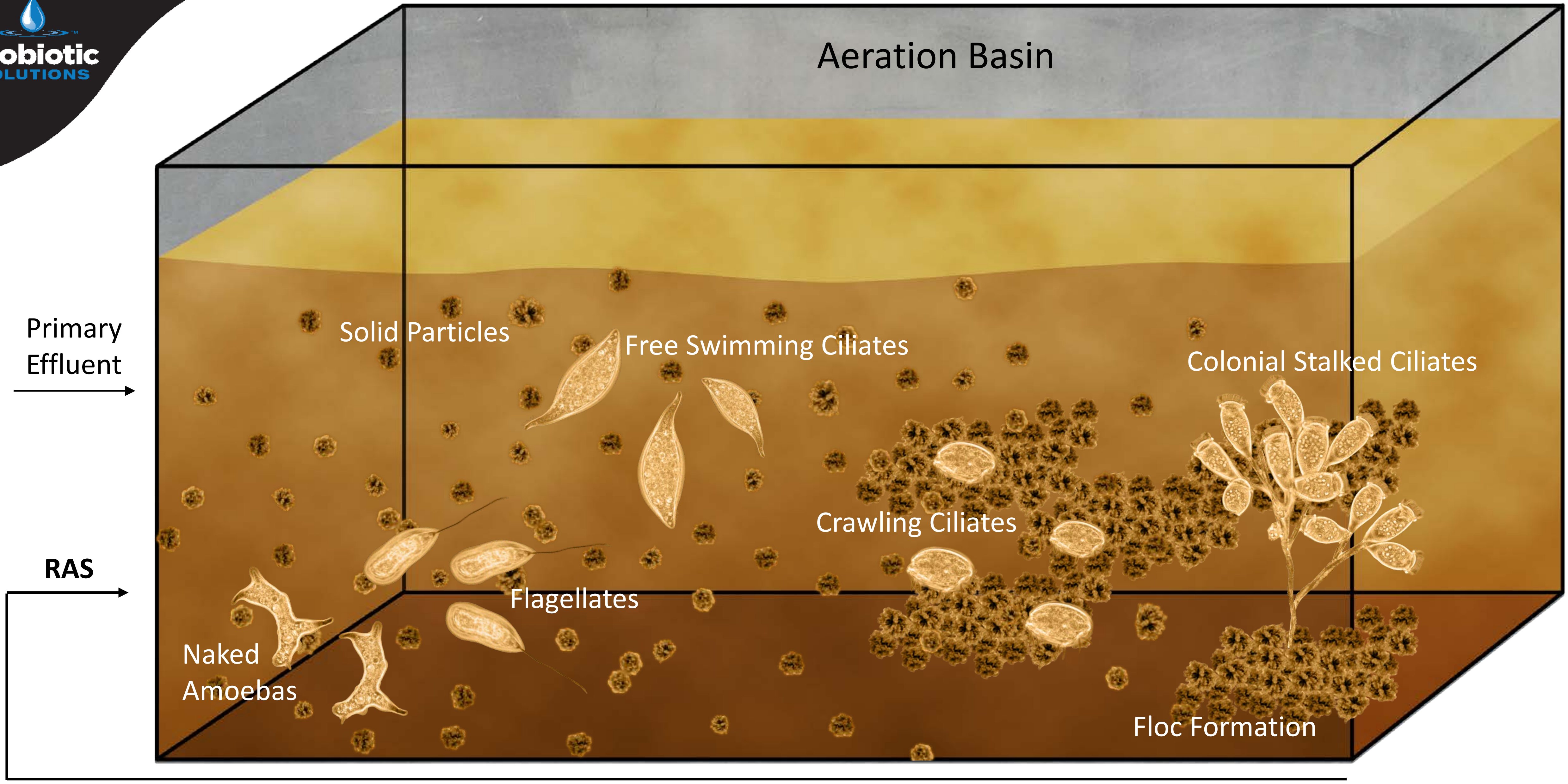
- Microbial growth occurs in stages
  - Lag phase: preparatory stage for division, hour to days
    - Bacillus sp. (BOD-eating bacteria) 20–30 mins.
    - Nitrifiers 22–48 hours
  - Log phase: exponential growth phase, cells are the most vulnerable at this stage
  - Stationary phase: maximum population density is reached
    - Death rate = growth rate
  - Death phase: rate of death exceeds the rate of growth
    - Endogenous phase: total mass of microorganisms begins to slowly decrease as the cells use up their stored reserves and begin to die

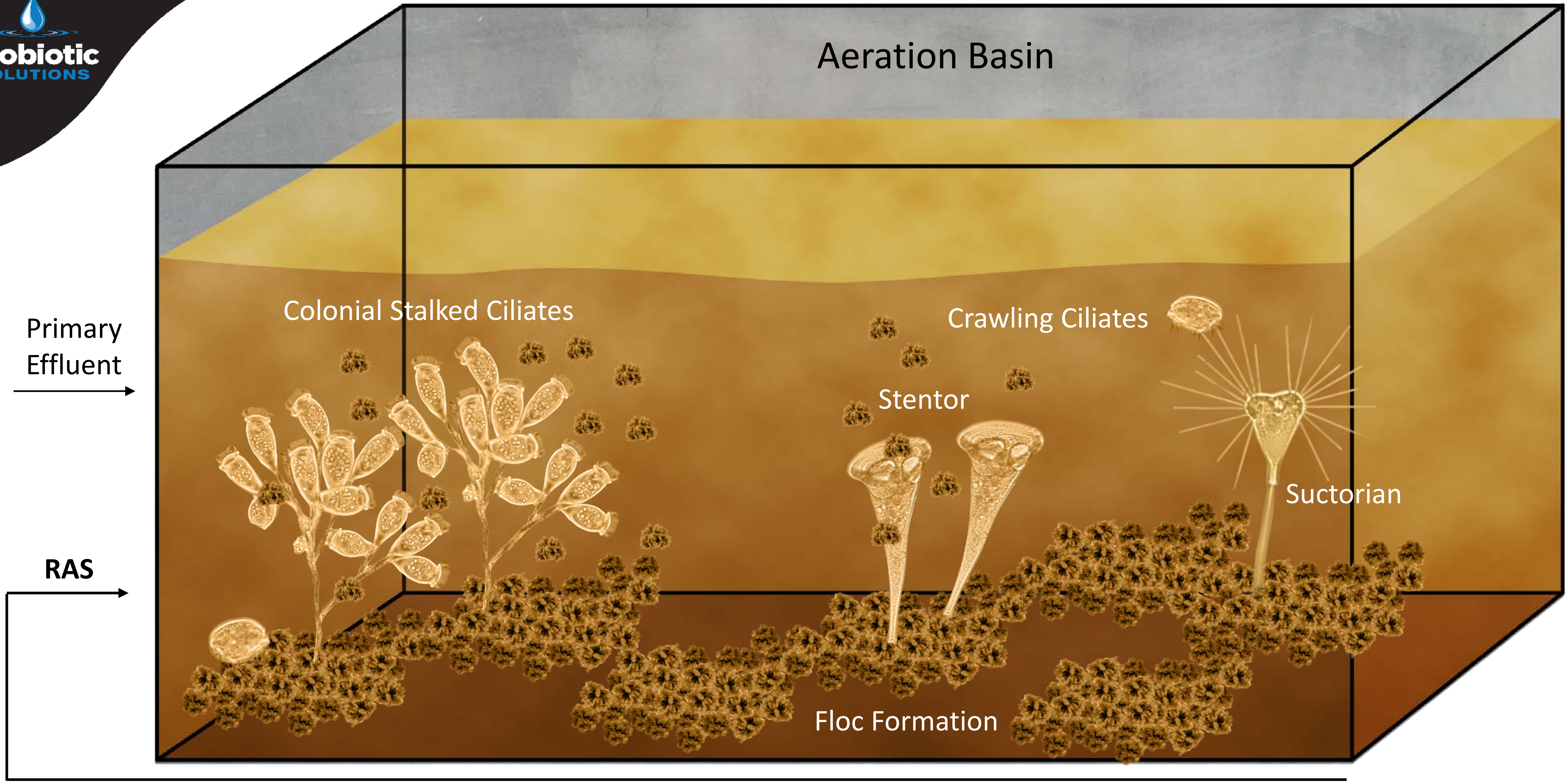

Stationary Phase
Lag Phase
Log Phase
Death or
Decline Phase
Logarithm of numbers of cells
Time

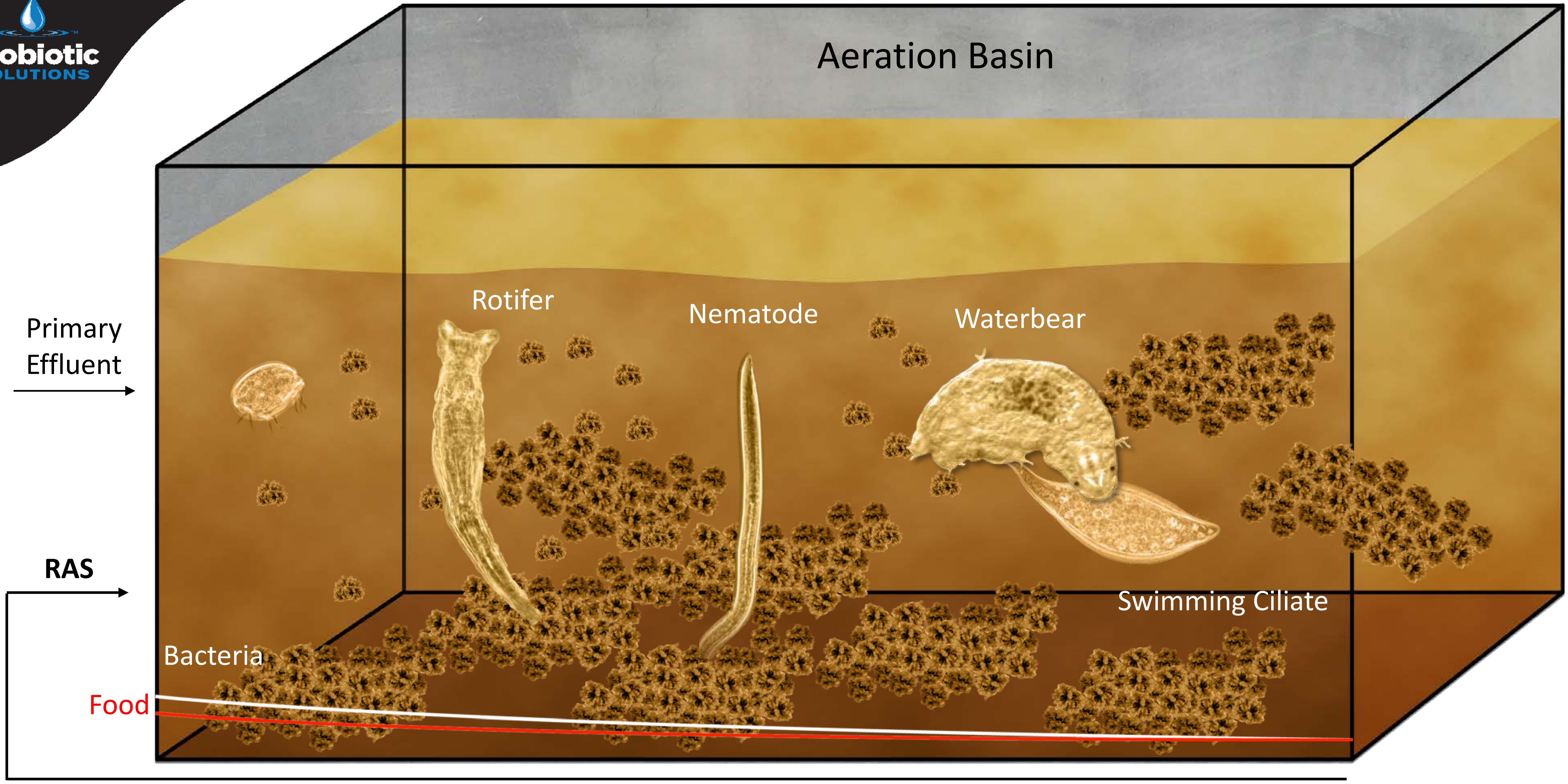


Logarithm of numbers of cells
Stationary Phase
Lag Phase
Log Phase
Death or
Decline Phase
Time

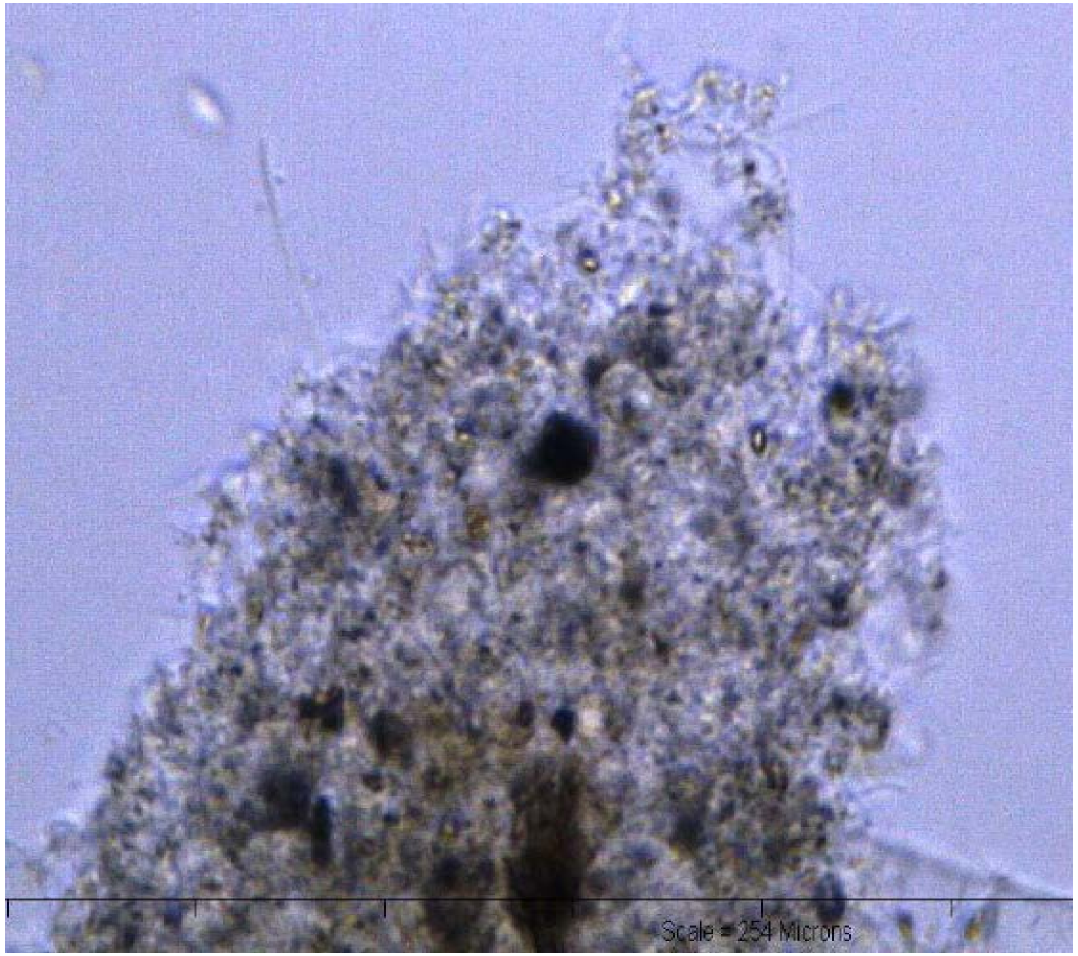

Aeration Basin
Food
Primary
Effluent
Solid Particles
Floc
RAS
Time


Aeration Basin
Primary
Effluent
RAS
Solid Particles
Free Swimming Ciliates
Colonial Stalked Ciliates
Crawling Ciliates
Flagellates
Naked
Amoebas
Floc Formation


Aeration Basin
Primary
Effluent
RAS
Colonial Stalked Ciliates
Crawling Ciliates
Stentor
Suctorian
Floc Formation


Aeration Basin
Primary
Effluent
RAS
Rotifer
Nematode
Waterbear
Swimming Ciliate
Bacteria
Food

Floc Morphology



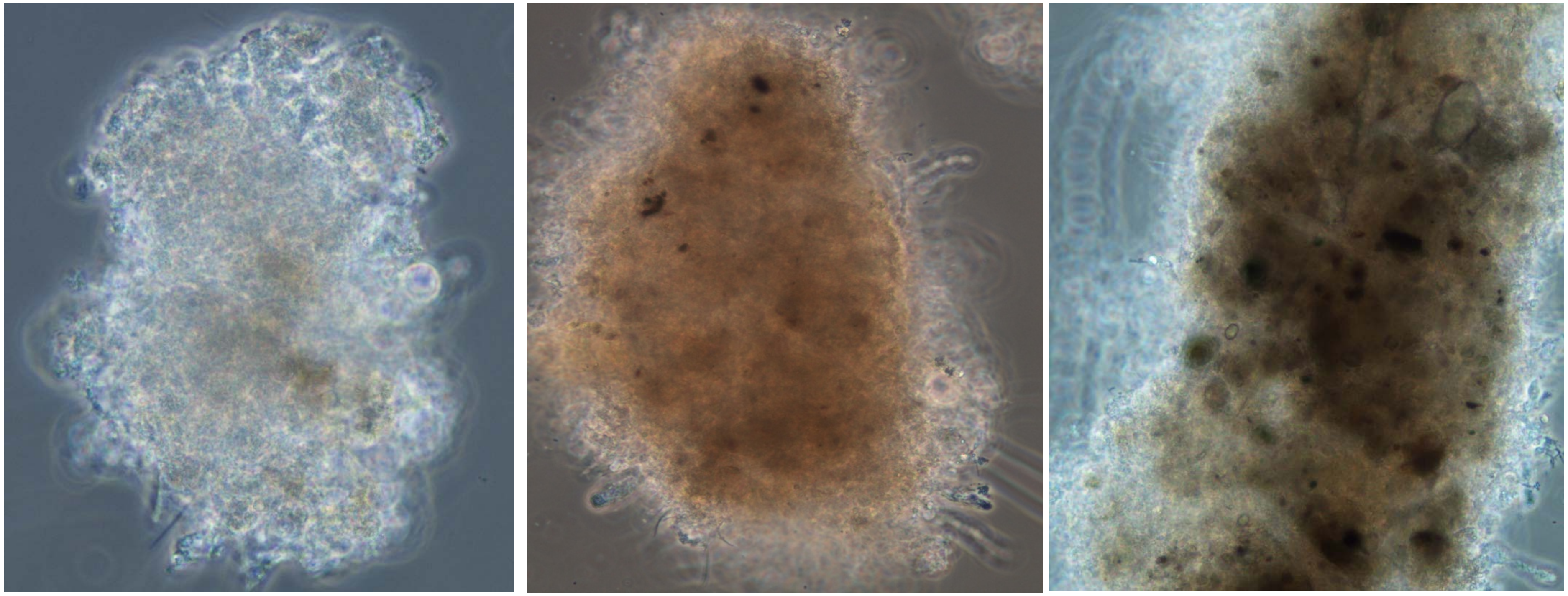
Floc Morphology

- Floc Morphology
  - Color
  - Shape and Structure
  - Size
  - Density
- Filamentous Bacteria
- Foam

Floc Morphology

- ***Color***: indicates the age of the biomass.
  - Clear indicates a very young biomass.
  - Golden brown indicates a healthy floc.
  - Black indicates the floc is turning anaerobic and running out of air or is older.
- ***Shape and Structures***: weak, lacy, open, diffuse, compact, firm, rounded
- ***Size***: regular or irregular, pin or large
- ***Density***: the more firm and compact a floc is, the better it will settle

Floc Morphology

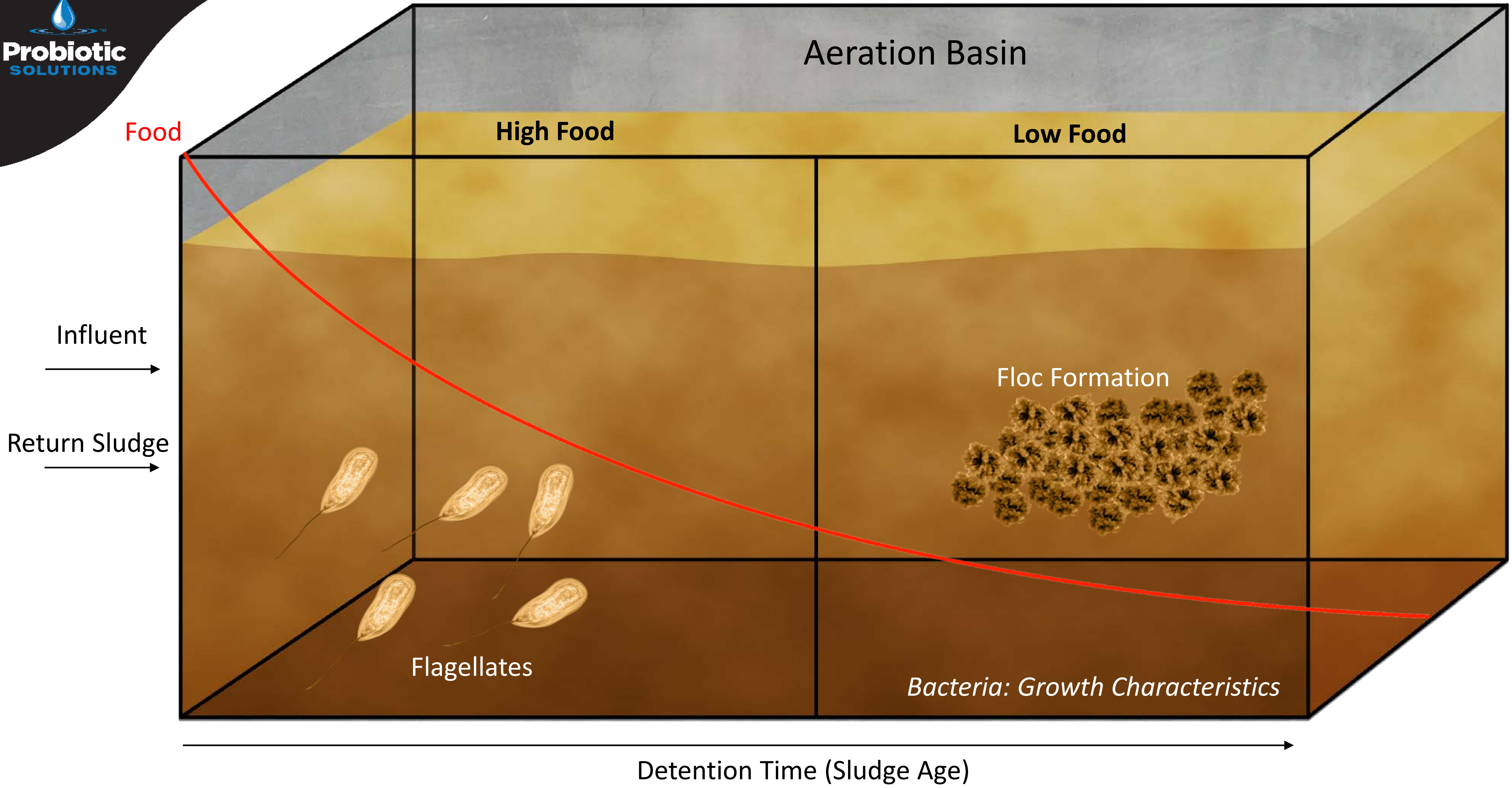


-5 0 +5

Toni Glymph Method

Floc Morphology

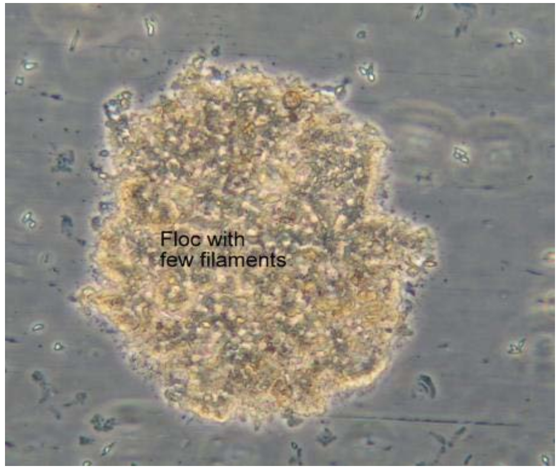
- Floc-formers generally react to negative situations by producing excess amounts of lipopolysaccharide.
- Non floc-formers generally form zoogleal masses in response to negative conditions.


Aeration Basin
Food
High Food
Low Food
Influent
Return Sludge
Floc Formation
Flagellates
Bacteria: Growth Characteristics
Detention Time (Sludge Age)

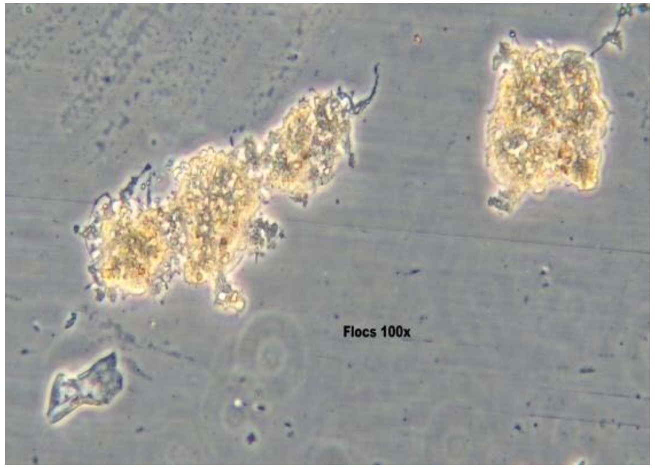
Filamentous Bacteria

- Several different ranking systems exist: Richard’s, Eikelboom’s, etc.
- ***Typical classifications***
  - – None to few
  - – Some
  - – Common
  - – Very common (where operational issues can appear)
  - – Abundant/Excessive

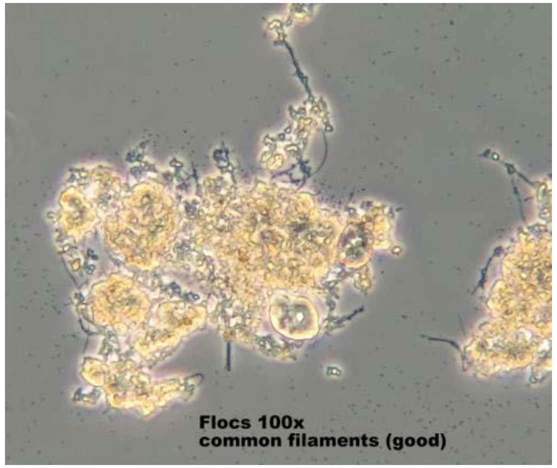
Filamentous Bacteria Ranking Examples



None to Few



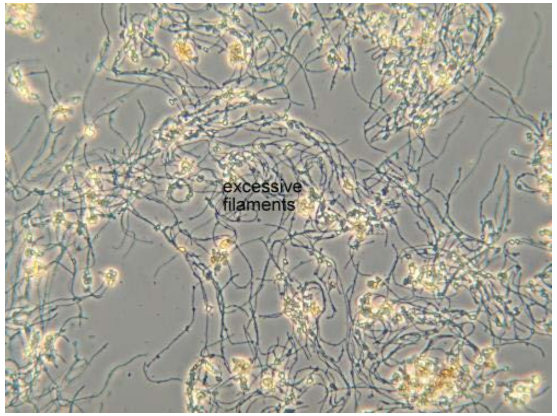
Some



Common

Very Common

Abundant



Excessive

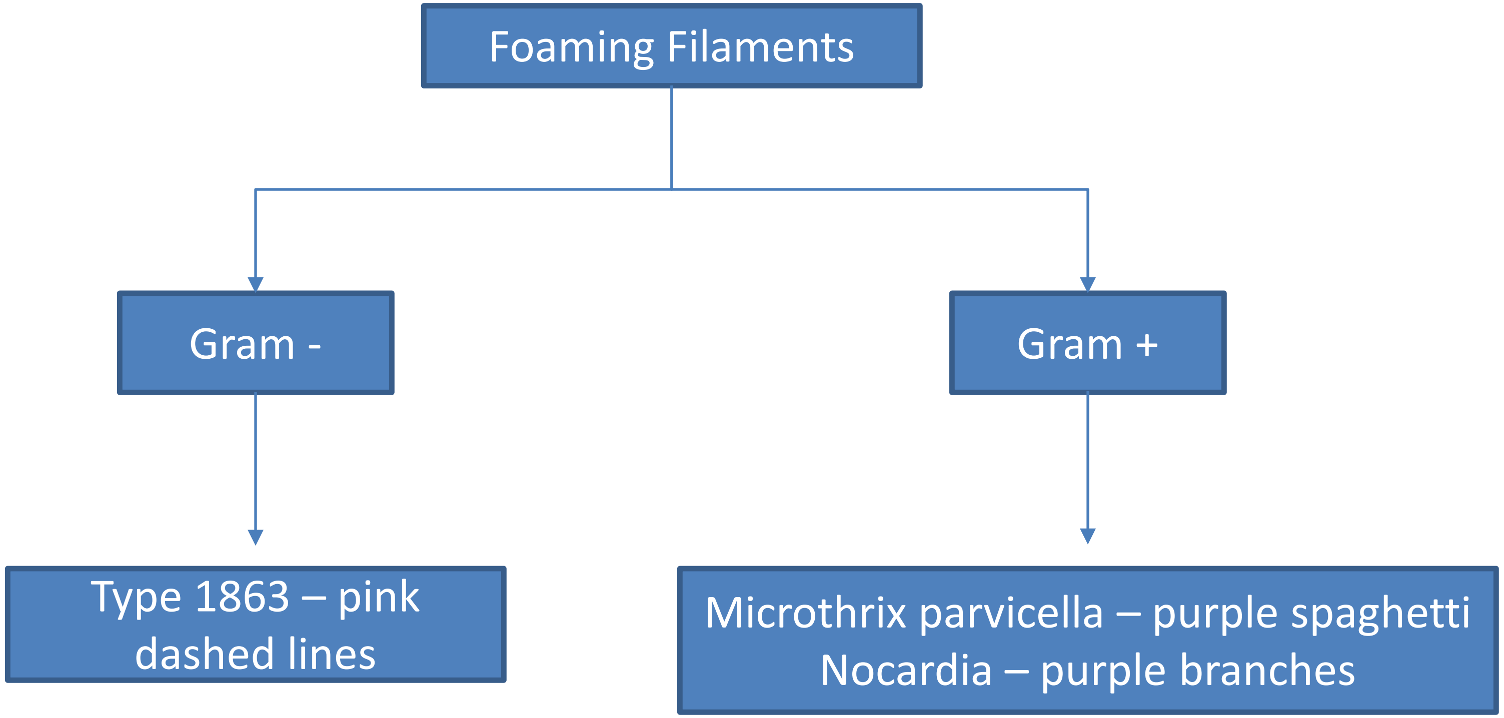
Filamentous Bacteria

- What you need to know when filamentous bacteria show up:
  - Influent load (BOD or COD)
  - pH
  - DO
  - Sludge age
  - Aeration basin F/M
  - Mixed liquor suspended solids
  - Temperature

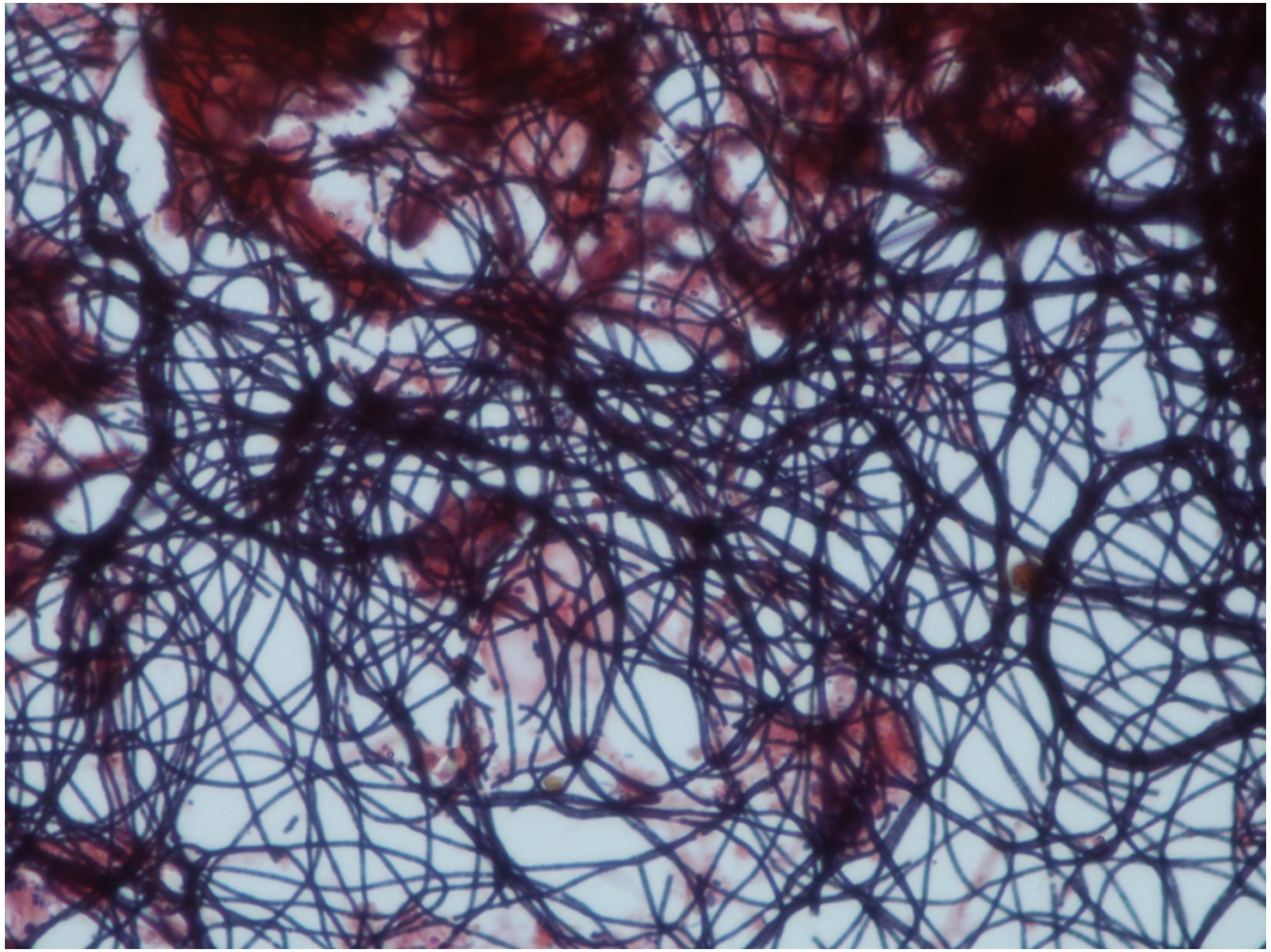
Filamentous Bacteria

- The SVI or 30-minute settling tests
  - Identify a problem but may not necessarily tell you what the problem is.
    - Filamentous bacteria
    - Excess zooglea
    - Slime bulking
- There are three main filaments that are responsible for the majority of the foaming in activated sludge treatment systems.
  - All prefer FOG and low F/M conditions

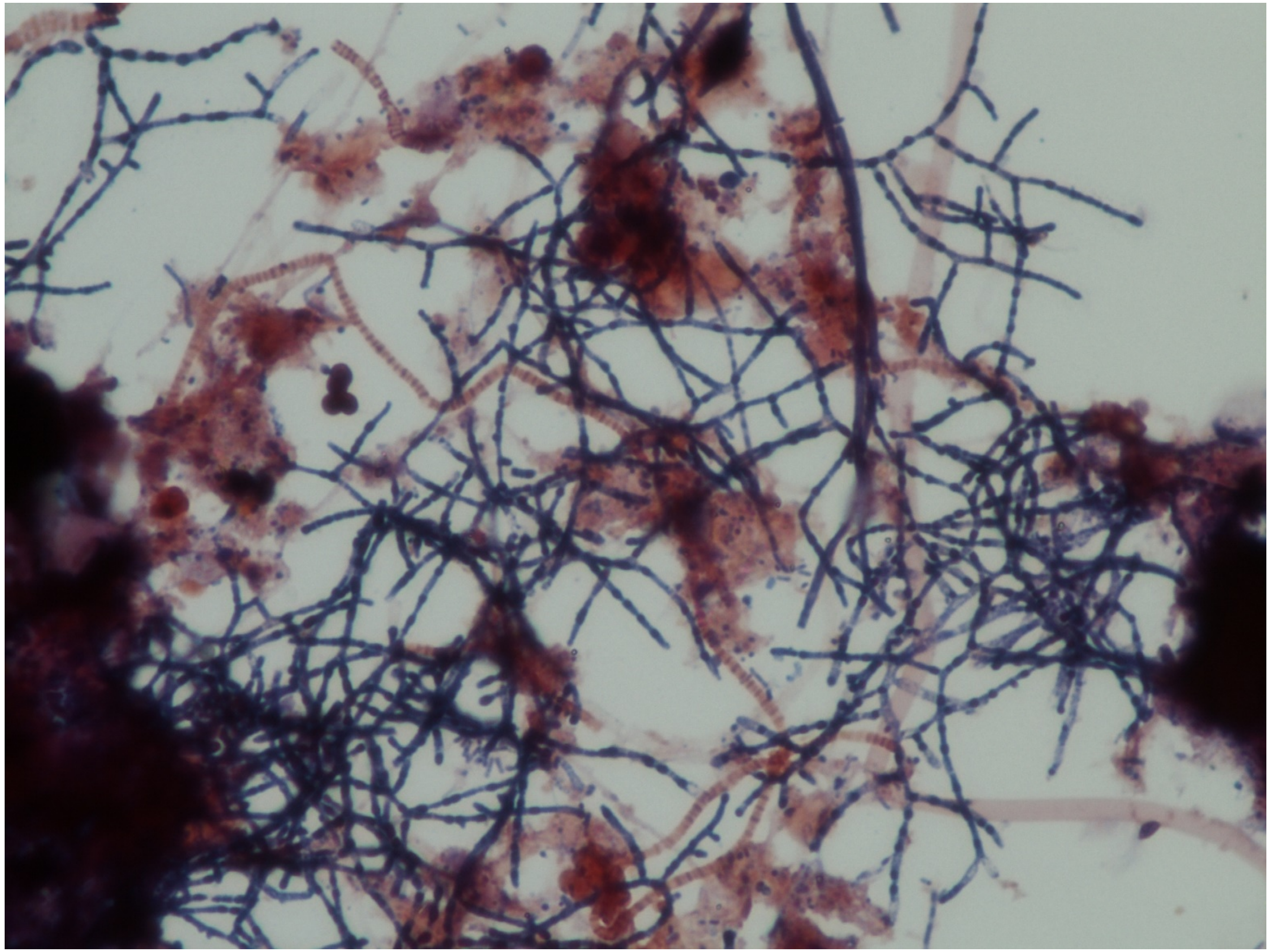
Filamentous Bacteria



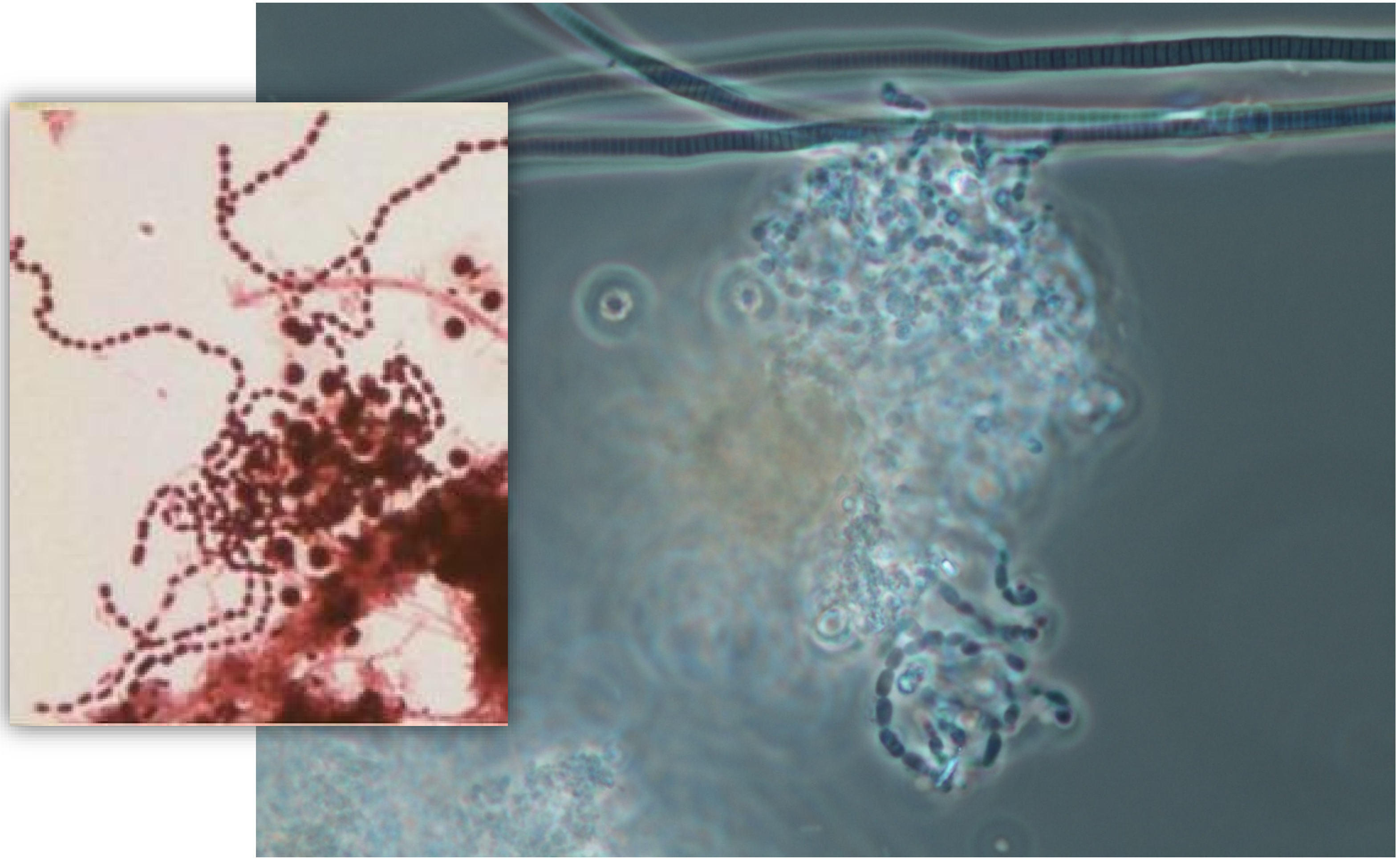
Microthrix Parvicella



Nocardia



Type 1863



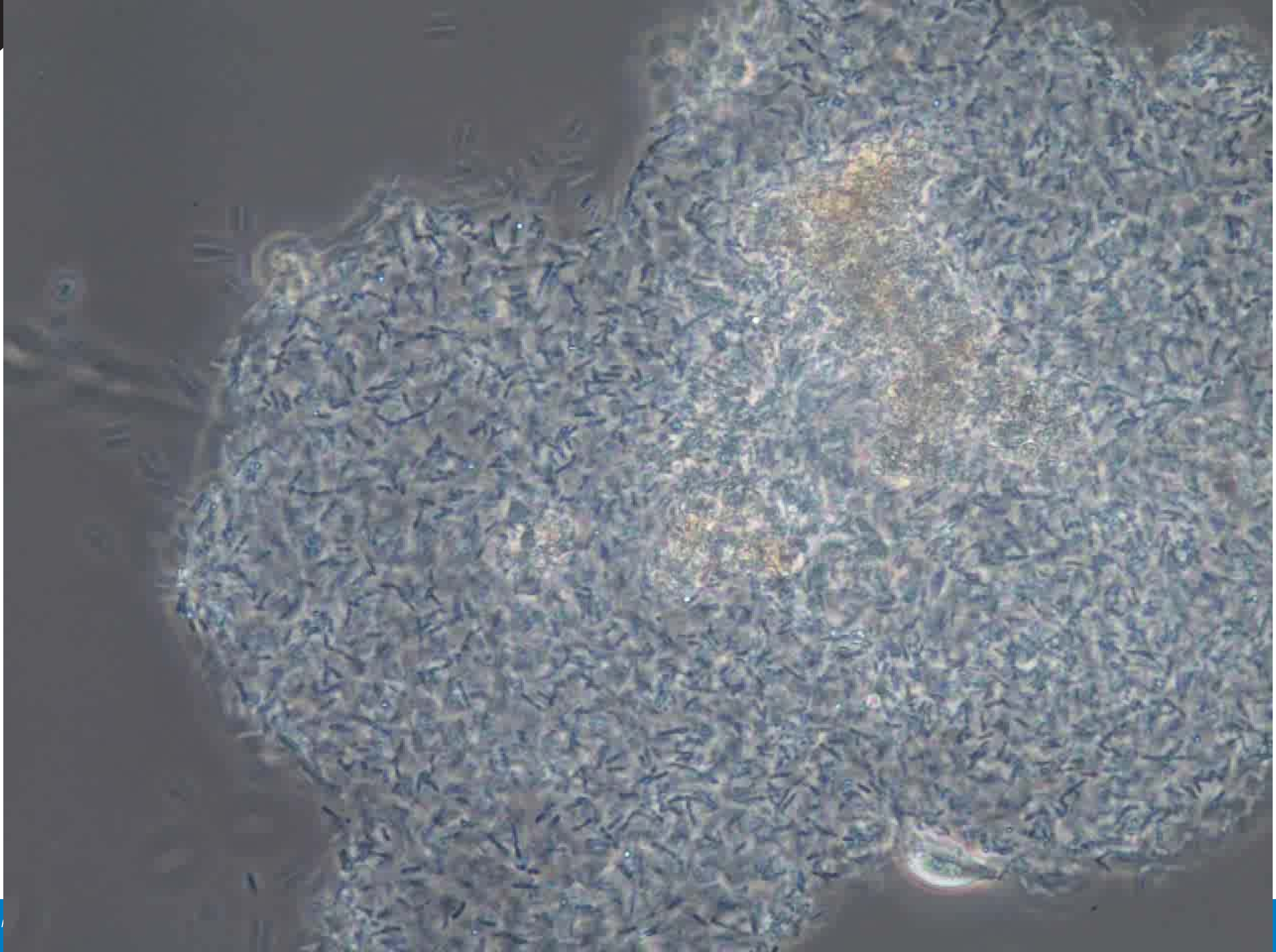
Probiotic SOLUTIONS

Bulking Filamentous Bacteria

Filament Name	Characteristics	Cause
Sphaerotilus natans	Sheath; round-ended rod cells, false branching, Gram(-)	Insufficient DO for the applied organic loading
Halicomenobacter hydrossis	Sheath difficult to detect; thin straight; Gram (-)	Low DO, low F/M; nutrient deficient conditions
Thiothrix I & II	Sheath; "barrel-shaped" cells; stores sulfur granules; Thiothrix Type I & II; Type I is twice the size as Type II, Gram (-)	Septic wastes; waste deficient in nitrogen; excess organic acids
Type 0041	Sheath; square-shaped cells; Gram-variable; attached growth	Low F/M; nutrient deficient conditions
Type 0675	Sheath; square-shaped cells; Gram-variable; attached growth, slightly smaller than type 0041	Low F/M; nutrient deficient conditions
Type 1701	Sheath; thin, round-ended rod cells, attached growth, Gram (-)	Low DO
Type 1851	Sheath; sparse attached growth; rectangular-shaped cells; grows in bundles	Low organic loading
Type 021N	Discoid-shaped cells; "stacked hockey pucks", round sulfur granules; slight reaction to Neisser stain	Septic wastes; waste deficient in nitrogen; excess organic acids
Beggiatoa	Motile; slowly gliding; stores sulfur granules	Septic wastes; waste deficient in nitrogen; excess organic acids; organic overload
Type 0914	Rectangular cells with rectangular-shaped sulfur granules	Septic wastes; waste deficient in nitrogen; excess organic acids; organic overload

Zooglea

- Zooglea
  - Responsible for sludge bulking
  - Polysaccharide slime
  - High F/M ratio
  - pH is usually lower in MLSS
  - May also be an indication of nutrient deficiency (nitrogen or phosphorus)
- Fixes
  - In the MLSS, the pH can be increased to above pH 7.
  - Nutrient addition is usually recommended.



Foam

- ***Foam***: colors can be indicators of operational issues
  - White: system start-up or possible excessive detergents in treatment waters

Foam *(Cont'd)*

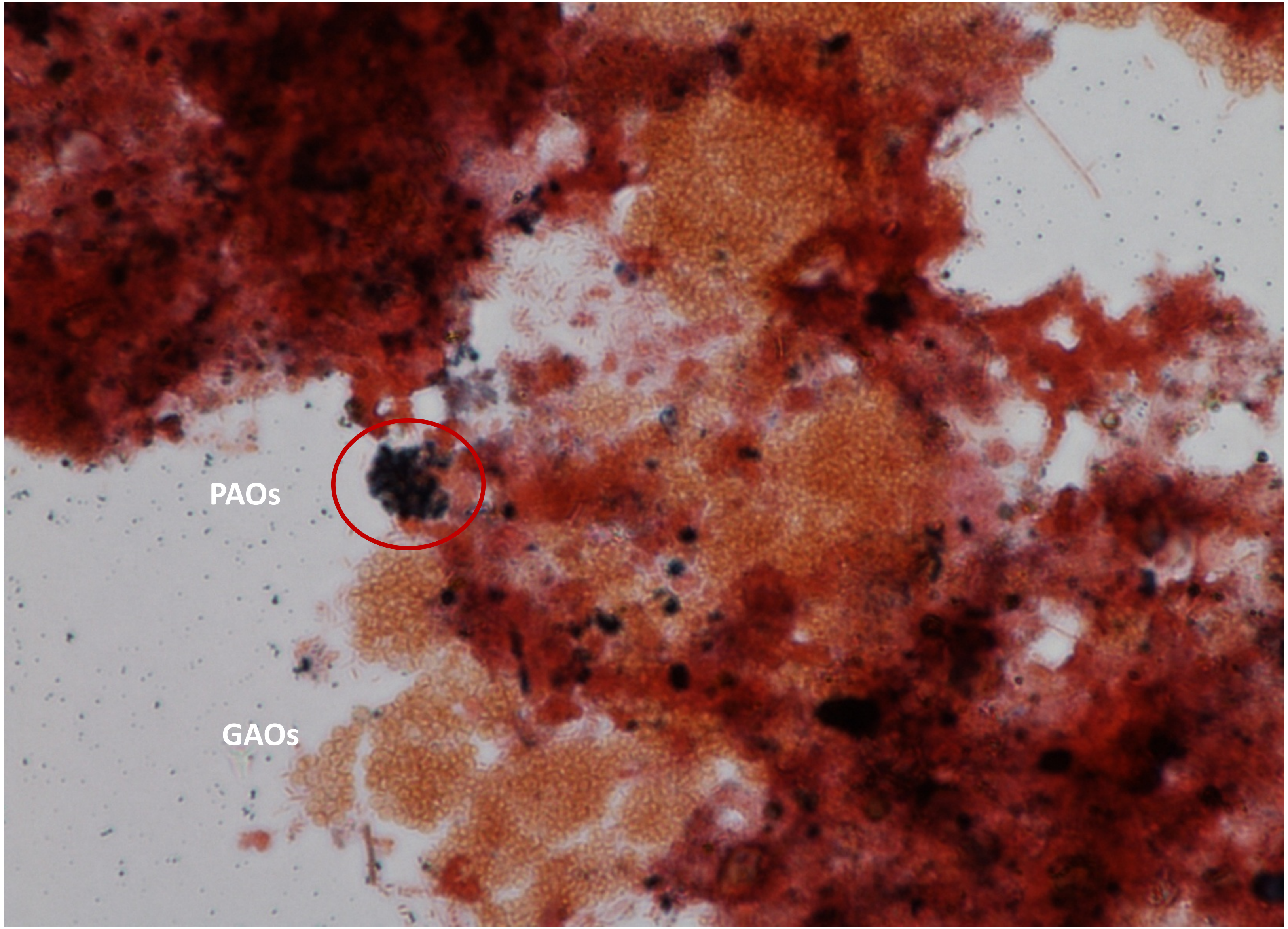
– Grey (ash): excessive fines from recycled systems
– Brown: filamentous, also called Nocaridia foam, others are Microthrix or Type 1863

Chlorination

- Chlorine is used to break and or damage filiments that extend above the wastewater
  - The trick: not damaging organisms within the floc and not the wastewater itself
  - Can make non-filamentous worse: e.g., slime bulking, zooglea bulking, or poor floc development
- While chlorination reduces bulking issues, if you reduce the chlorination the filaments will regrow rapidly!
  - Underlying bulking issues are left unresolved

New Perspective

- Tracking PAOs and GAOs to predict WWTP behaviors.
  - Phosphorus (polyphosphate) Accumulating Micro-organisms (PAOs)
    - Aerobic phase of the process, PAOs are able to multiply and take up phosphate to replenish the supplies depleted in the anaerobic phase.
  - Glycogen-Accumulating Organisms (GAOs)
    - GAOs are capable of taking up the often-limited VFA substrates from EBPR systems anaerobically; however, GAOs do not contribute to P removal.
  - Tracking the quantity of PAOs and GAOs in your system provides a way to anticipate toxicity issues.
    - Each system is unique.


PAOs
GAOs

New Perspective

- Precursor to toxicity is changes in the dissolved oxygen uptake rate (DOUR)
  - Unique to each system
  - **DOUR, in mg O_2/L/hr = (DO_initial – DO_final) * 60 / Length of test in minutes**

Summary

- Why
- When
- Where
- Questions To Be Thinking About
  - What am I looking for?
  - What do I know once I have looked?
  - When should I send the sample out?

References

- *Microbiological Examination of Water and Wastewater*, Lewis Publishers, Maria Csuros, Csaba Csurus, 1999
- *Handbook of Microscopic Examination of Sludge*, 1983, Eikelboom, D.H. and Van Buijsen, H.J.J.
- Minnesota Pollution Control Agency, Phosphorus Treatment and Removal Technologies https://www.pca.state.mn.us/sites/default/files/wq-wwtp9-02.pdf
- *Causes and Control of Activated Sludge Bulking and Foaming, Second Edition*, D. Jenkins, M.G. Richard and G. Daigger, Lewis Publishers, Boca Raton, FL, 1993. http://www.dec.ny.gov/chemical/34373.html
- Website http://group1micropara.weebly.com/classification.html
- **Toni Glymph,** Senior Environmental Microbiologist at Metropolitan Water Reclamation District, Chicago

Probiotic® SOLUTIONS™

Thank You

Publication No. PS-160928-01