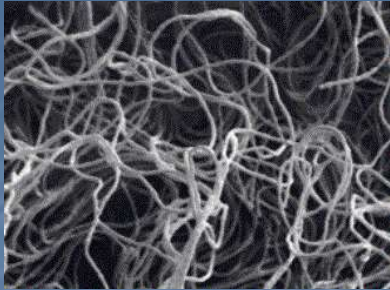




Identification & Control of Filamentous Bacteria

Toni Glymph, Wastewater Microbiologist
ToniGlymph@msn.com



Filamentous Bacteria

Sludge Bulking



Filamentous Bacteria

Sludge Foaming



FILAMENTOUS BACTERIA

- In the activated sludge treatment system, bacteria may occur singly, or in small chains or clumps.
- Shifts in the activated sludge environment such as changes in pH, dissolved oxygen, nutrients etc. will often cause a change in the behavior of the bacteria.

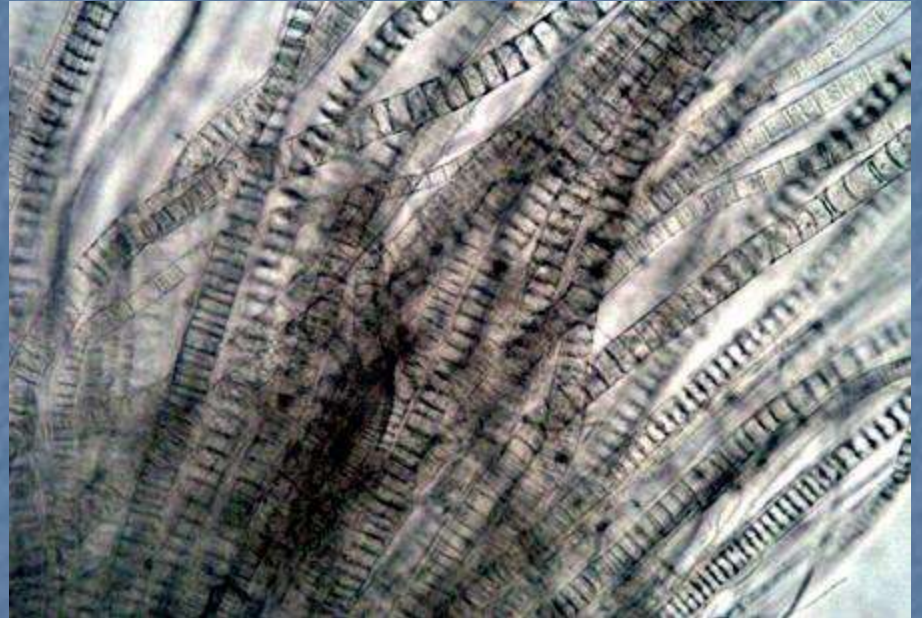
FILAMENTOUS BACTERIA

- In stead of single cells, small chains or clumps, the bacteria will begin to form longer "chains" which develop into filamentous bacteria.

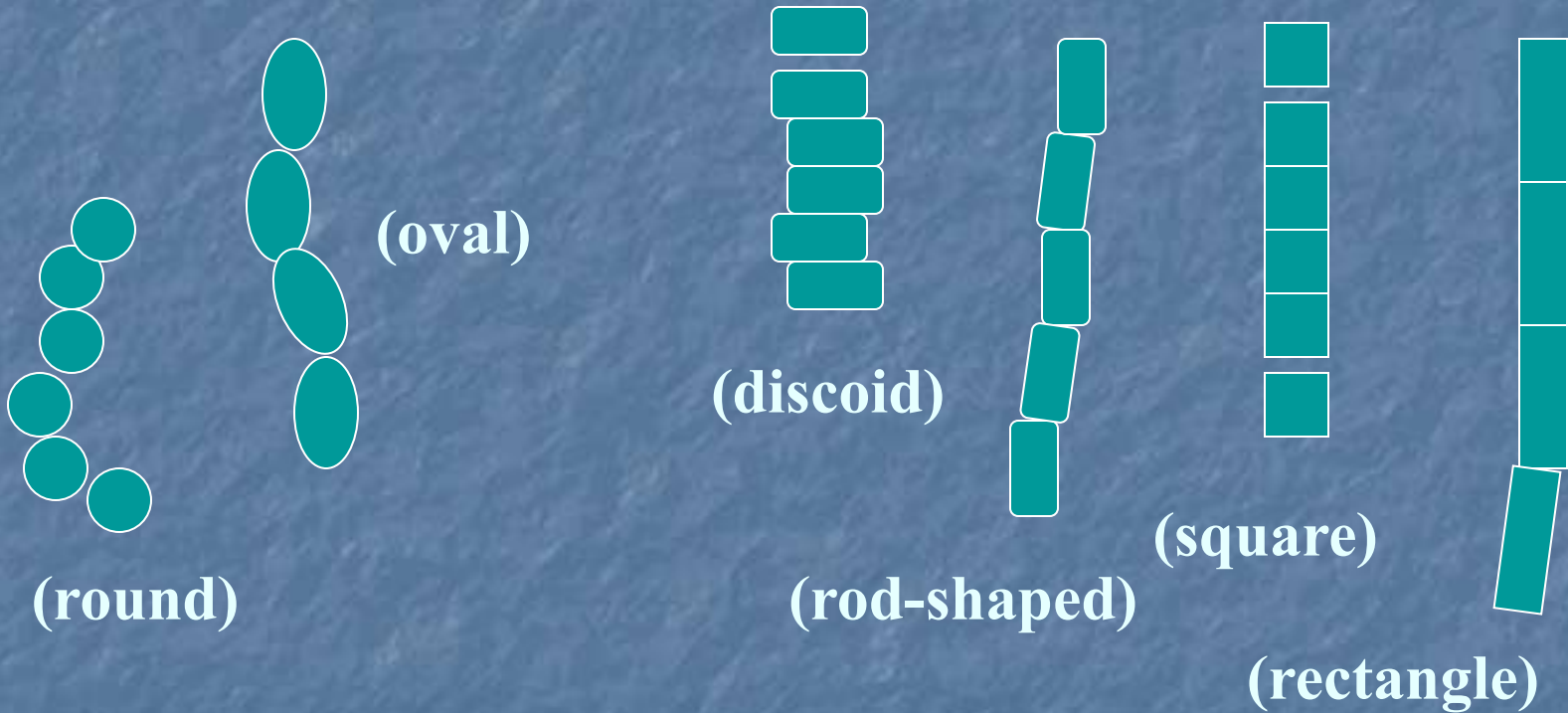


Filamentous Bacteria

- Round bacteria will form a chain with other round bacteria, square with square, rectangle with rectangle etc.



FILAMENTOUS BACTERIA



Cocci

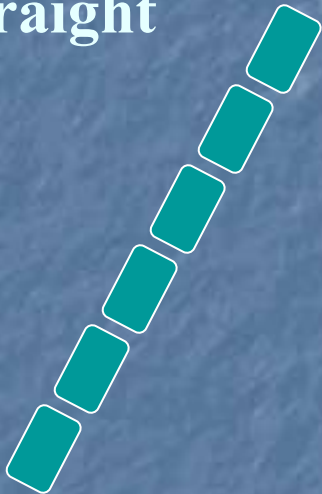
Bacillus

Typical Observation

- Filament Shape
- Filament Size
- Cell shape
- Cell size
- Cell Septa
- Indentations
- Sheath (present/absent)
- Branching (true/false)
- Epiphyte (attached growth)
- Motility

Typical Observation: *Filament Shape*

Straight



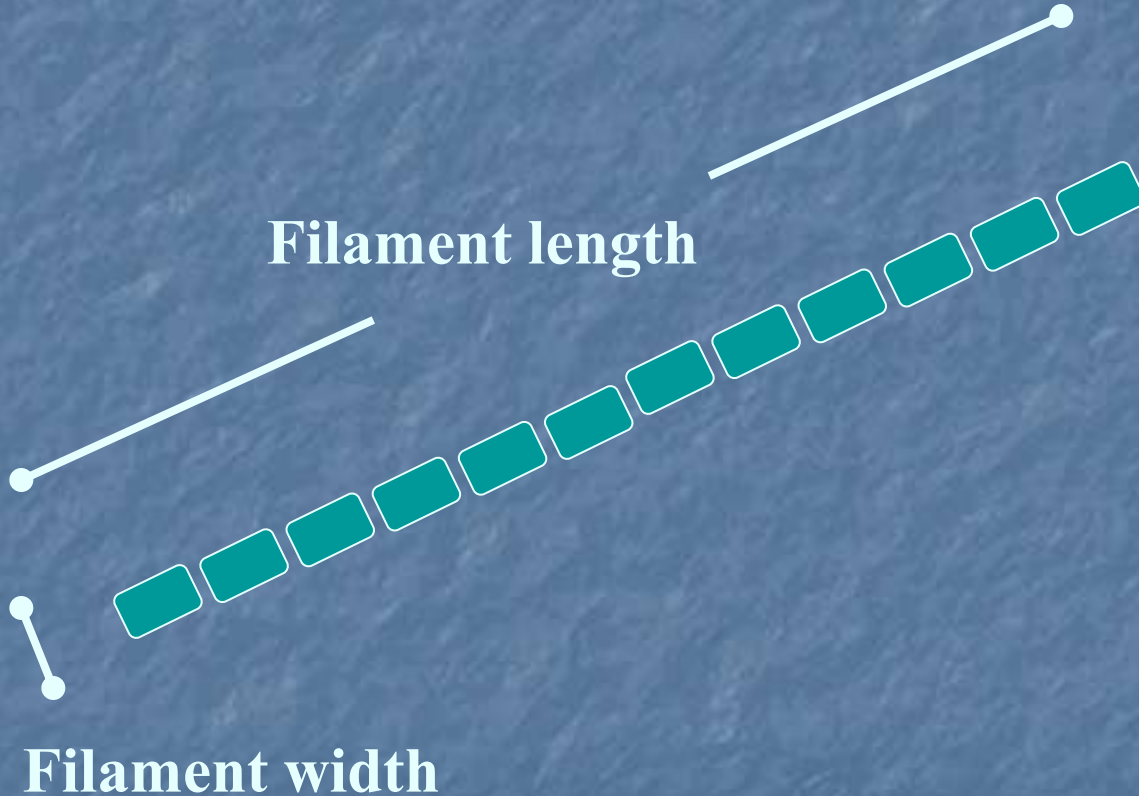
**Smoothly
Curved**



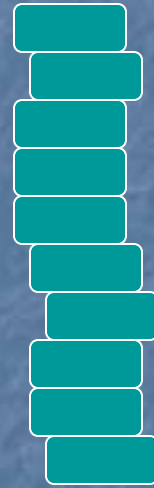
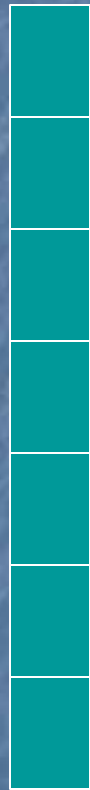
Irregular



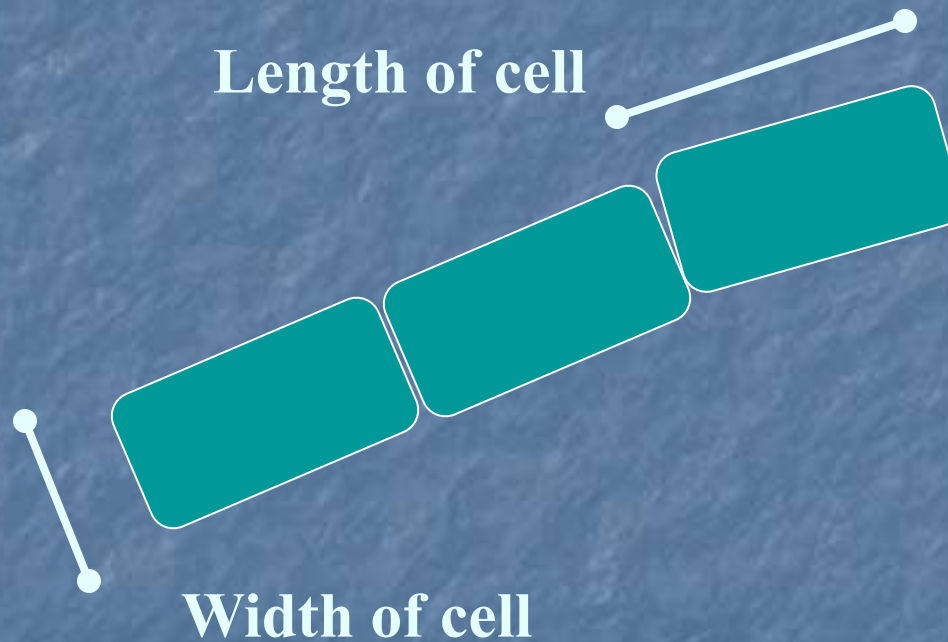
Typical Observation: *Filament Size*



Typical Observation: *Cell Shape*



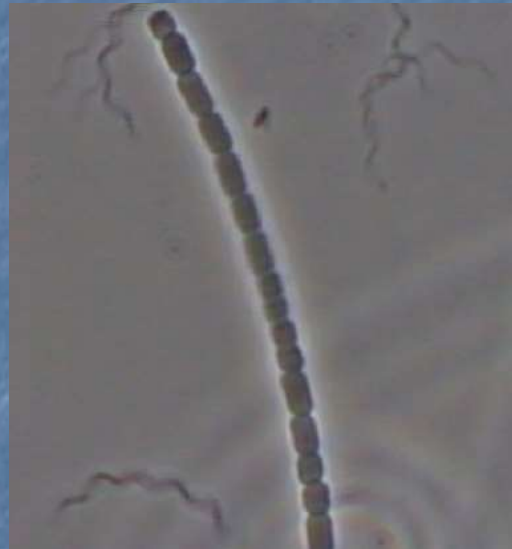
Typical Observation: *Cell Size*



Typical Observation: *Cell Septa*



**Cell septa with
no indentation**

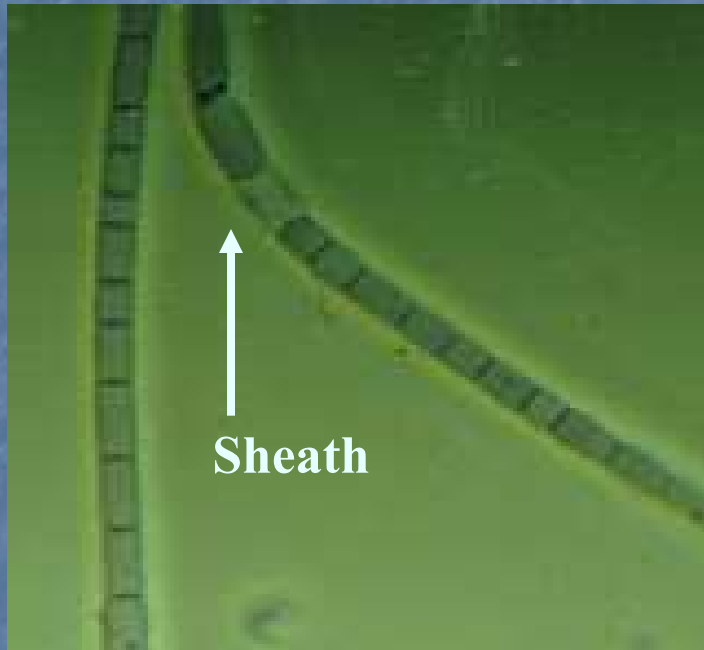


**Cell septa with
indentation**



**Cell septa not
clearly seen**

Typical Observation: *Sheath*



Individual cells within a sheath

Typical Observation: *Branching*



True branching
Fungus



**False
branching**
S. natans

Typical Observation:
Epiphyte (Attached Growth)



Typical Observation: *Motility*

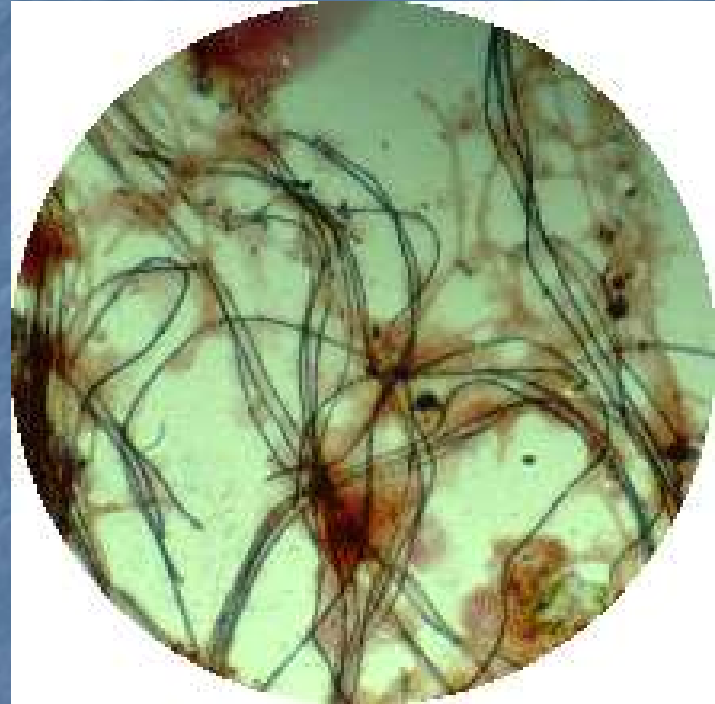


Microscope Requirements

- Phase contrast Microscope
- 10X or 20 X objective
- 40 X objective
- 100 X (oil immersion lens)

Stains

- Gram Stain
- Neisser Stain

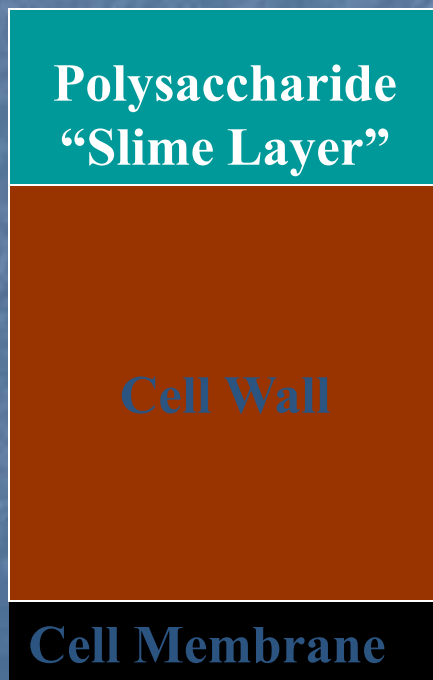


Gram Stain

- Separates bacteria into two categories
- Determined by the bacteria cell wall
 - Gram (+) bacteria cell wall retains the purple color
 - Gram (-) bacteria cell wall loses the purple color
- Gram positive bacteria are purple
- Gram negative bacteria are pink

Gram Stain

Gram positive



Gram negative



Gram Stain



Gram (+) *Nocardia* sp.



Gram (-) *Type 1701*

Gram Stain

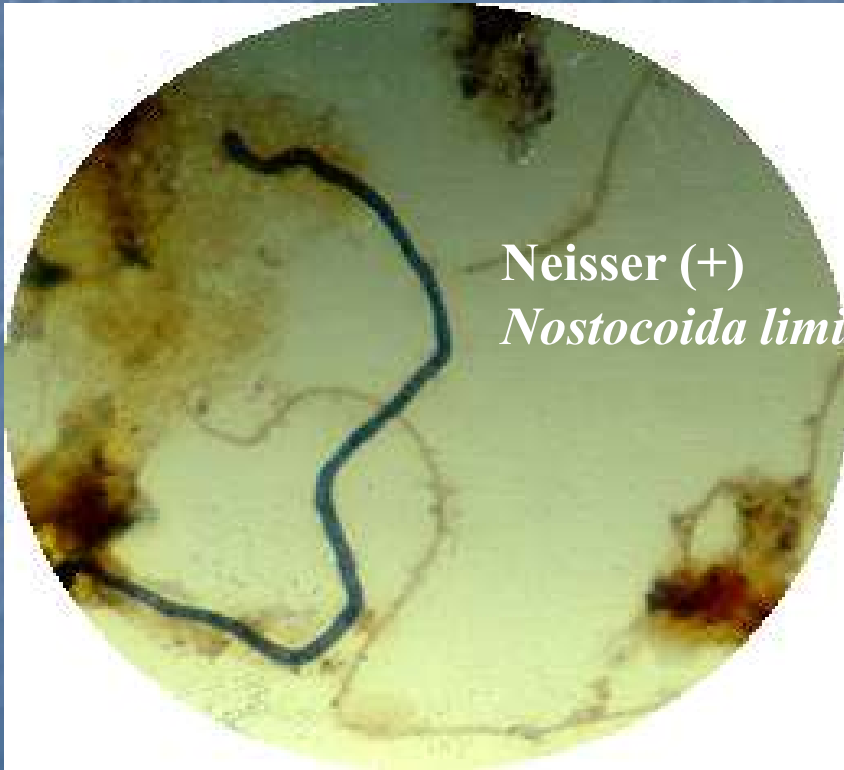


Gram variable Type *0041*

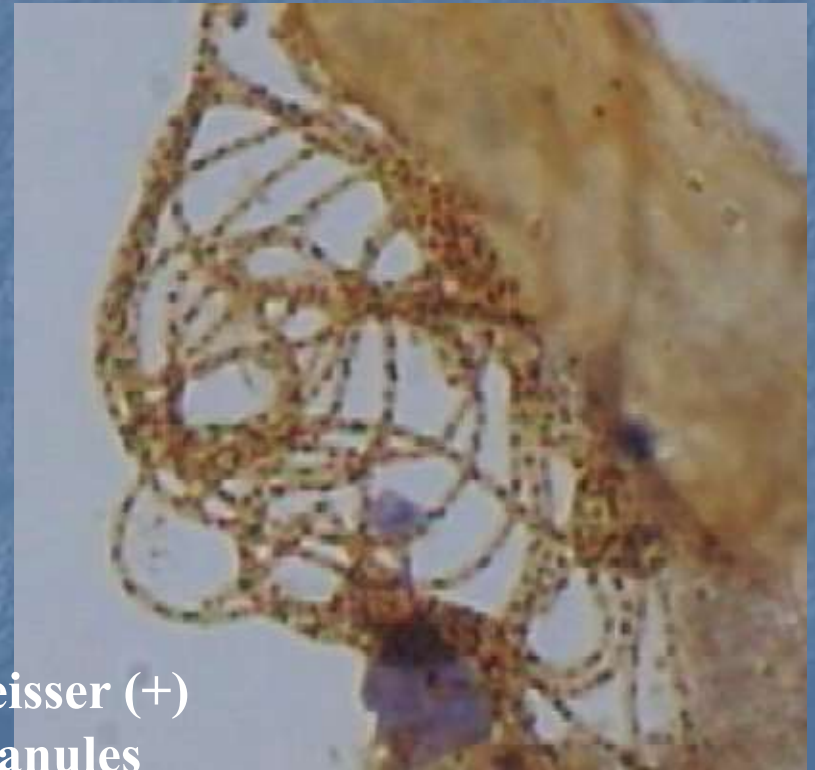
Neisser Stain

- Separates bacteria into two categories
- Distinguishes the bacteria which contain granules that accumulate polyphosphate
- Neisser (+) bluish color
- Neisser (-) brownish color

Neisser Stain

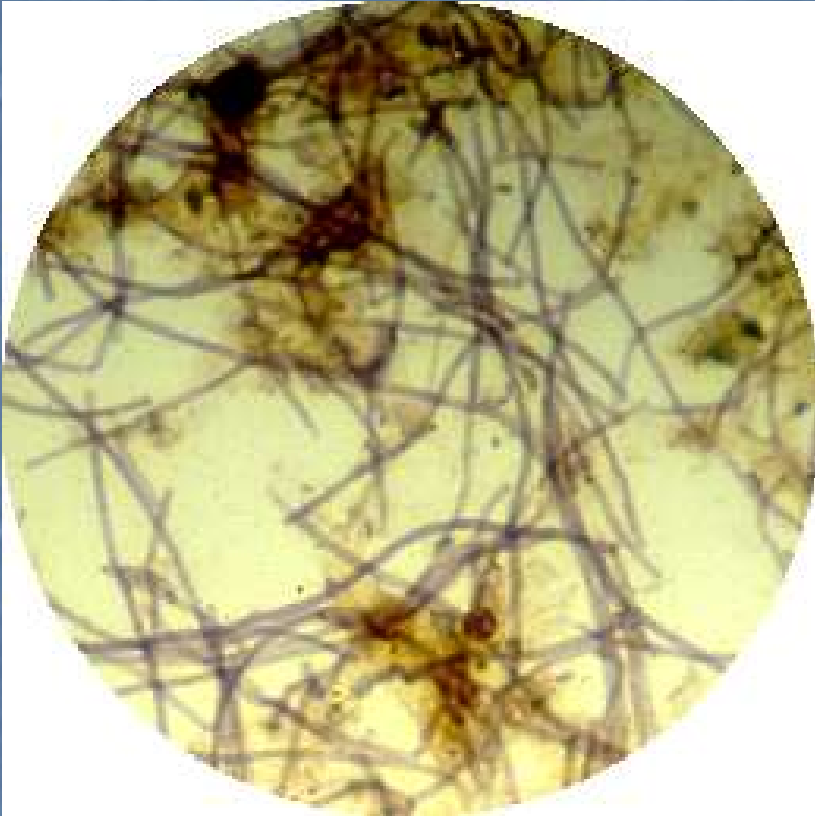


Neisser (+)
Nostocoida limicola



Neisser (+)
granules

Neisser Stain



Neisser (+) Type 0092



Neisser (+) Nostocoida limicola
and *Neisser (-) Thiothrix*

Slide Preparation

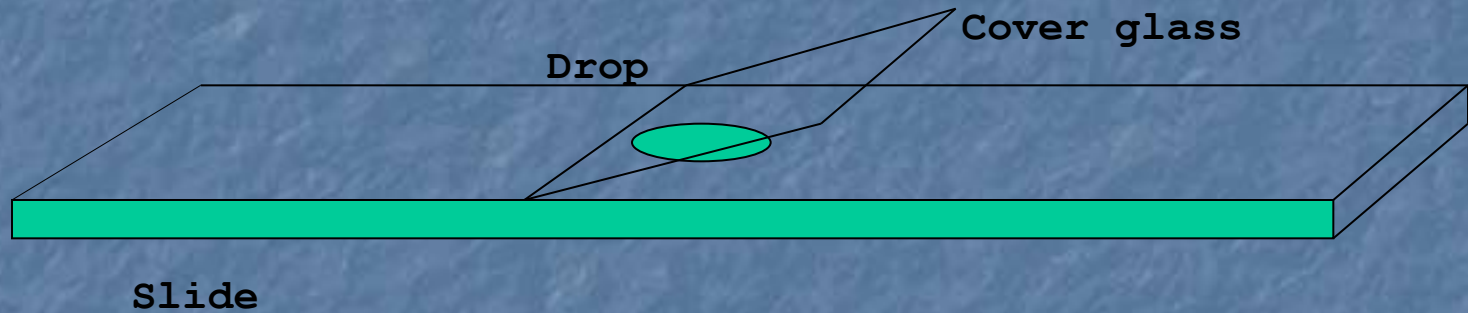
- Wet Mount
- Smear
 - Dried for staining

Slide Preparation:

Wet Mount

- A drop of a well mixed representative sample is place on a clean grease-free slide.
- A clean cover-glass is placed on top. Avoid entrapment of air as much as possible.
- The size of the drop is critical
- Use a pipette with a small opening

Slide Preparation: *Wet Mount*



Slide Preparation:

Wet Mount

- This allows the observer to view the microorganisms live in their environment.
- With a live sample, measurements can be taken and cell shape and size can be accurately determined.

Slide Preparation:

Smear

- Place a drop of sample on a clean, wax-free slide and smear across the slide.
- Let the slide air dry. DO NOT HEAT FIX!

Slide Preparation: *Smear*



Slide

Sample Collection

- For bulking sludge, collect samples from the discharge end of the aeration basin.
- For foaming sludge, collect a sample of the foam.

Filamentous Bacteria

- Most filamentous bacteria in Activated Sludge are associated with low F/M along with some other factor(s) that uniquely gives them an advantage over the other microorganisms.
- Discovering and changing what gives them the advantage will help control their dominance.

Filamentous Bacteria

A Simpler Approach

- Bulking Filaments
- Foaming Filaments

Filamentous Bacteria

Bulking Filaments

- Collect a sample of the mixed liquor from the discharge end of the aeration basin
- Make a smear
- Gram stain
- Neisser stain (optional)
- Wet Mount

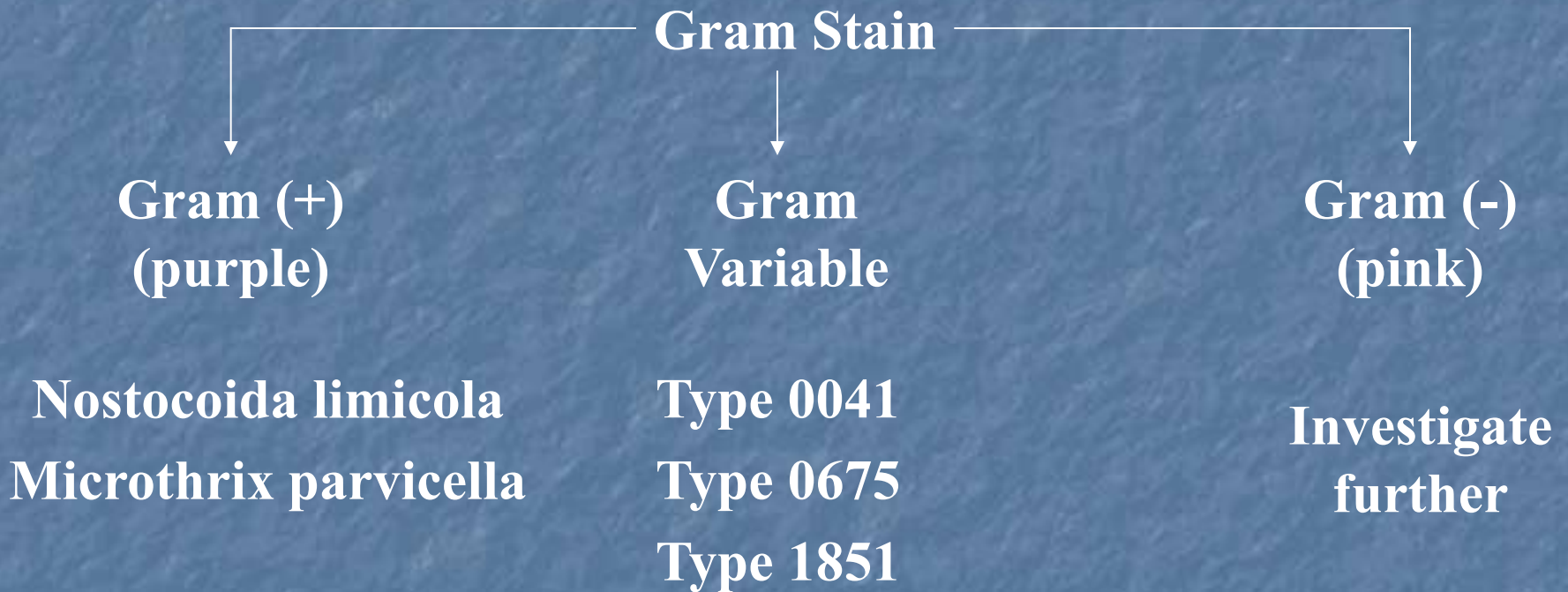
Filamentous Bacteria

A Simpler Approach

- Nostocoida limicola
- Sphaerotilus natans
- Haliscomenobacter Hydrossis
- Thiothrix I & II
- Microthrix parvicella
- Nocardia
- Beggiatoa
- Type 0041
- Type 0675
- Type 0092
- Type 1851
- Type 1701
- Type 021N
- Type 0914
- Type 0961
- Type 1863

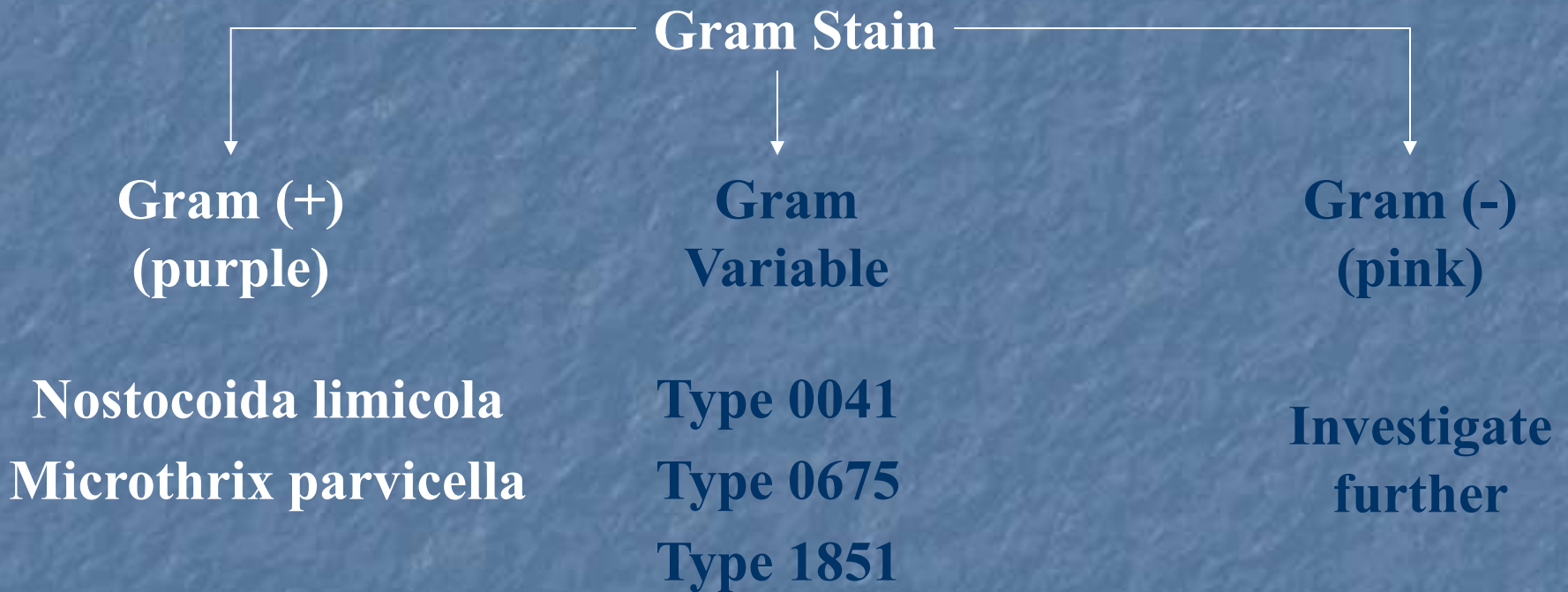
Filamentous Bacteria

Bulking Filaments



Filamentous Bacteria

Bulking Filaments



Bulking Filaments

Nostocoida limicola



Bulking Filaments

Nostocoida limicola



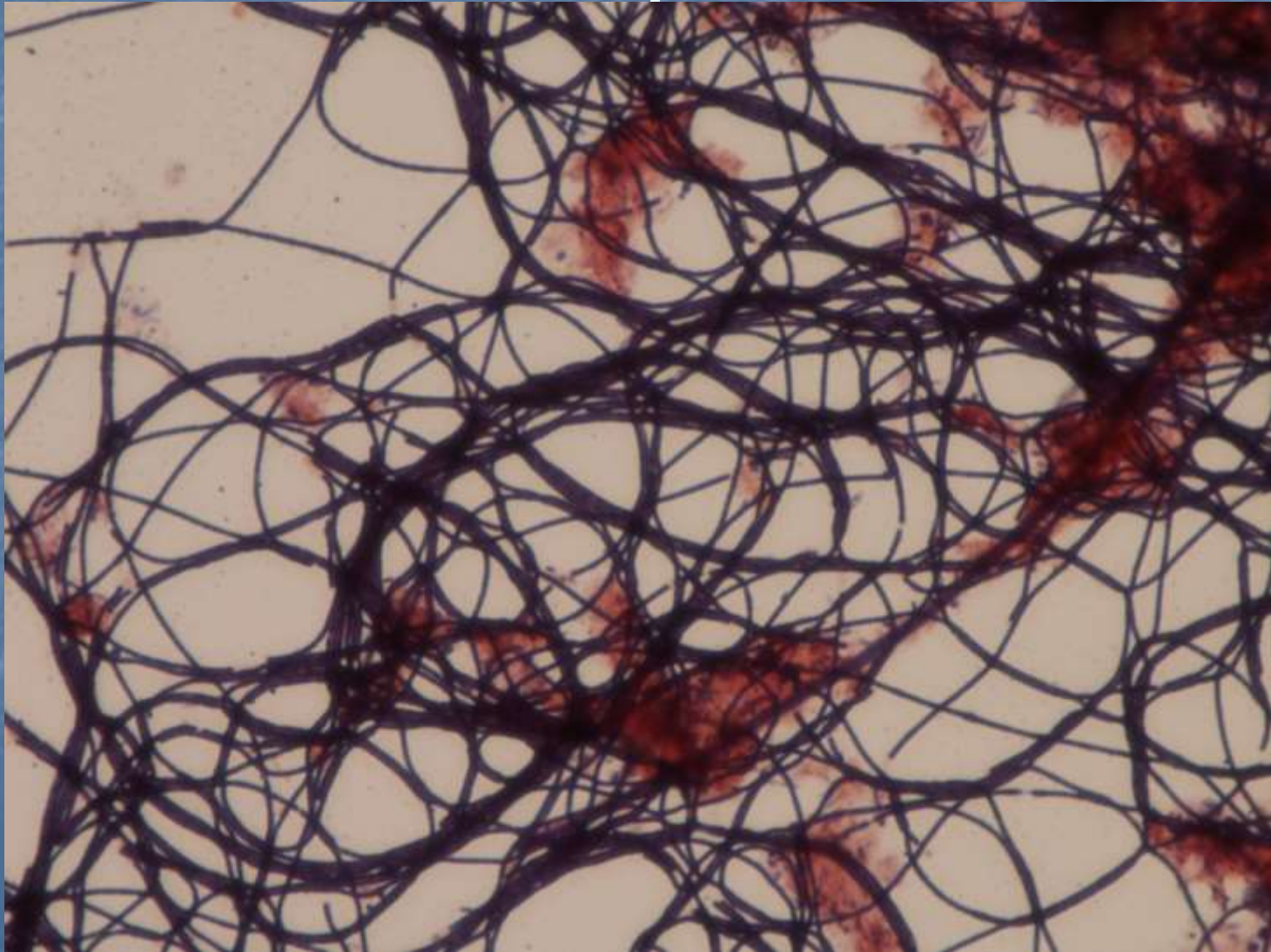
Bulking Filaments

Nostocoida limicola

- This filament is associated with
 - Low F:M
 - Starch containing wastes (potato processing)
 - More commonly found in industrial waste plants

Bulking Filaments

Microthrix parvicella



Bulking Filaments

Microthrix parvicella

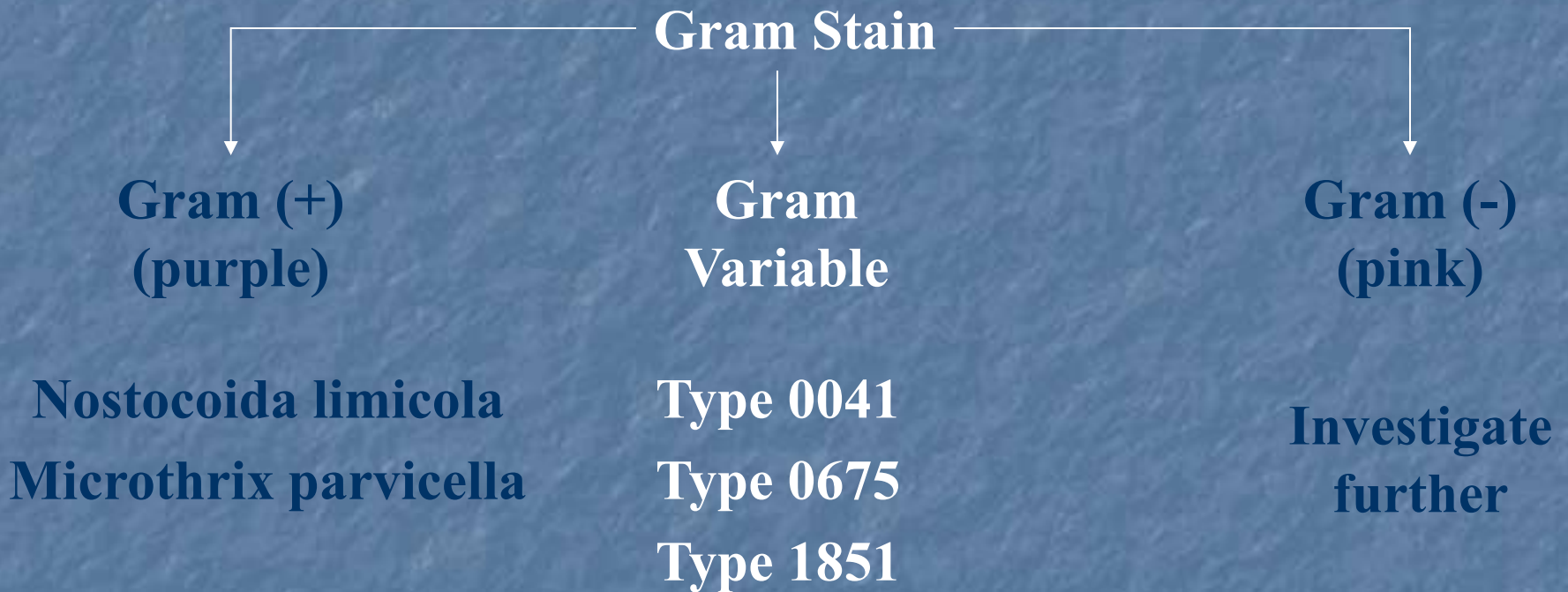
- This filament is associated with
 - Low F:M
 - Animal and vegetable greases, oils and fats
 - More common in colder temperatures
 - Also causes foaming

Filamentous Bacteria

Bulking Filaments

- *Nostocoida limicola*
- *Sphaerotilus natans*
- *Haliscomenobacter Hydrossis*
- *Thiothrix I & II*
- *Microthrix parvicella*
- *Beggiatoa*
- Type 0041
- Type 0675
- Type 0092
- Type 1851
- Type 1701
- Type 021N
- Type 0914
- Type 0961

Bulking Filaments



Bulking Filaments

Gram Variable

- Type 0041
- Type 0675
- Type 1851

Gram Variable

Type 0041



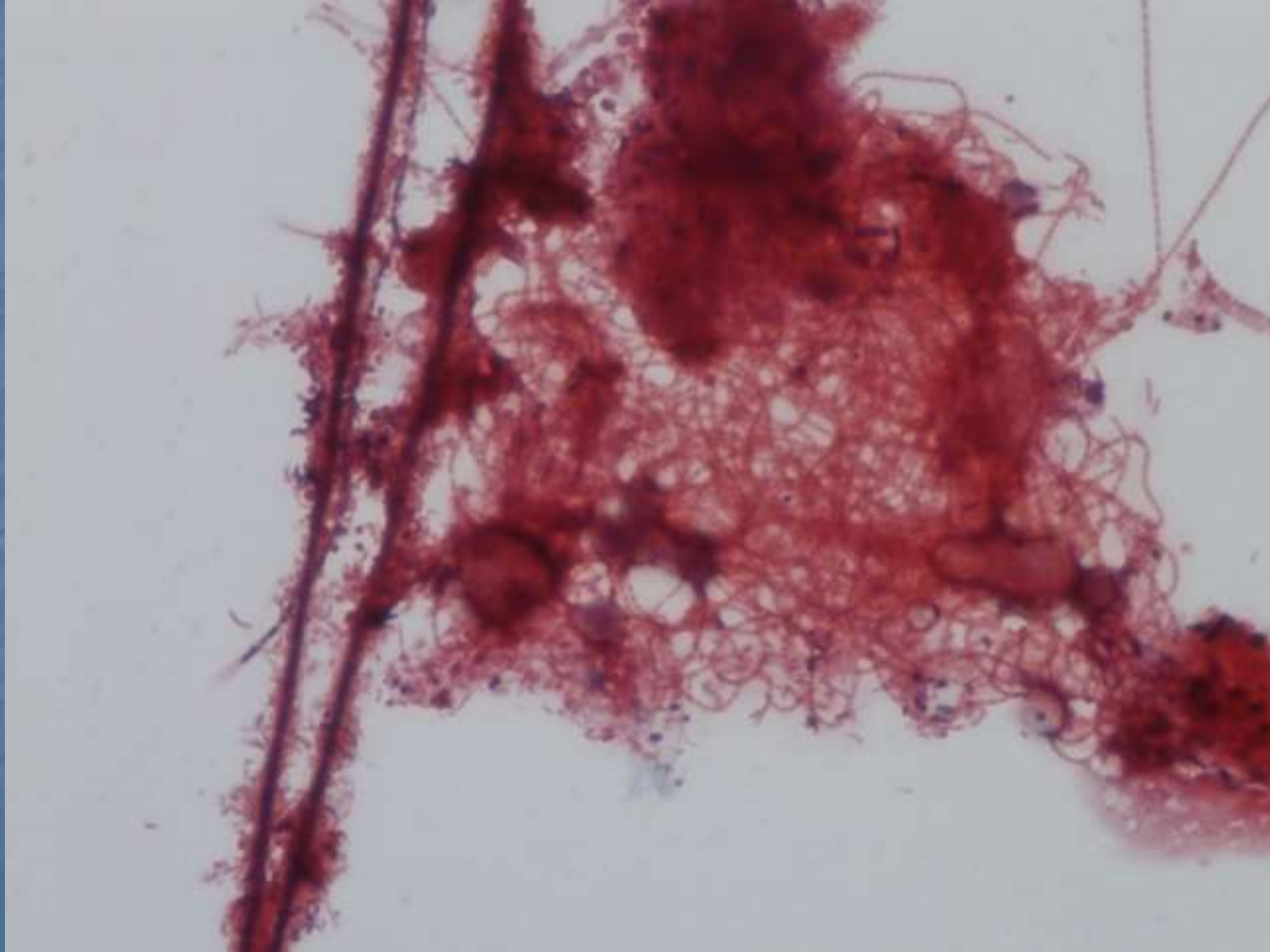
Gram Variable

Type 0041

- This filament is associated with
 - Low F:M
 - Nutrient deficient conditions

Gram Variable

Type 0675



Gram Variable

Type 0675

- This filament is associated with
 - Low F:M
 - Nutrient deficient conditions

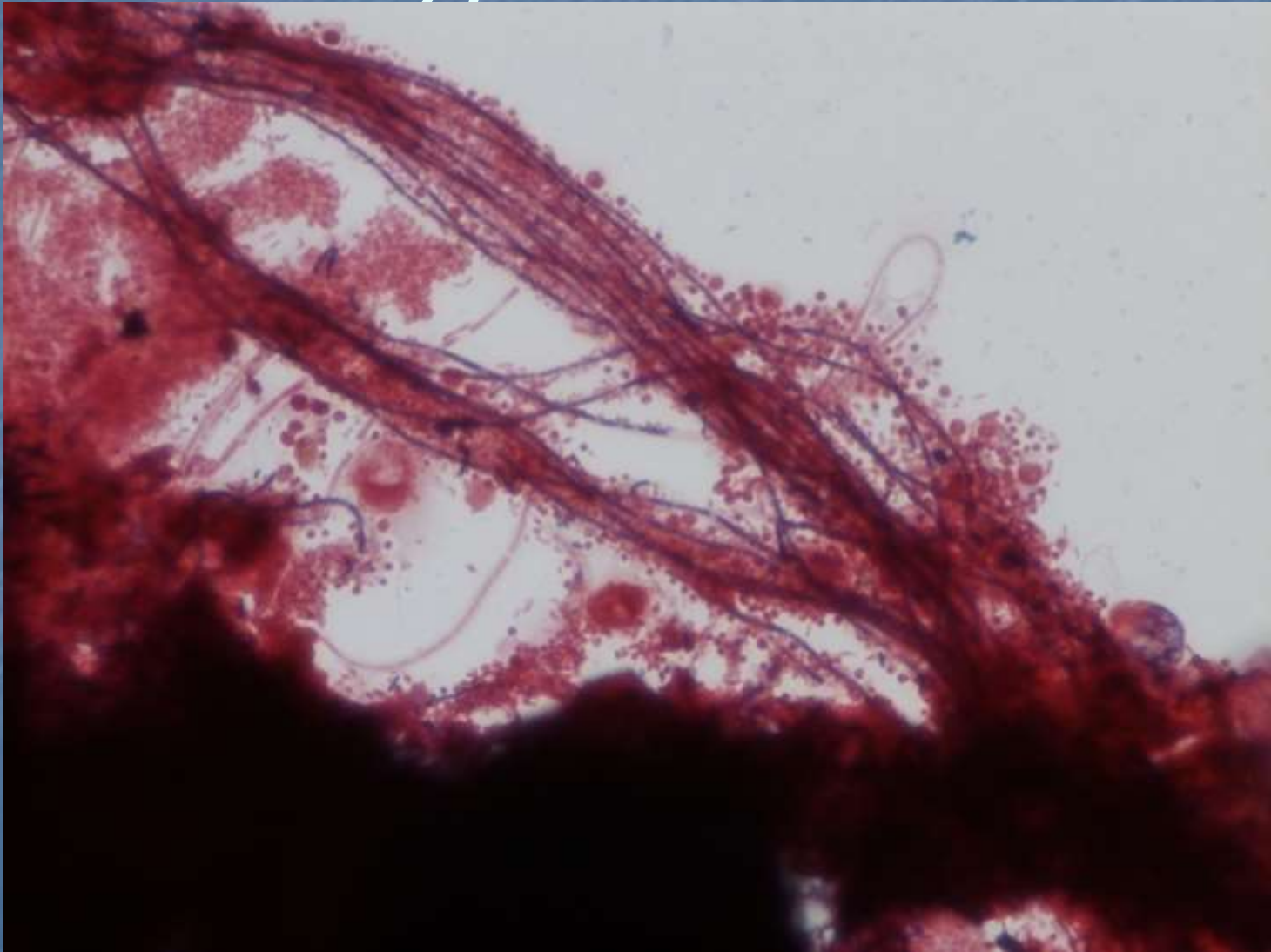
Gram Variable

Type 1851



Gram Variable

Type 1851



Gram Variable

Type 1851 Bundles



Gram Variable

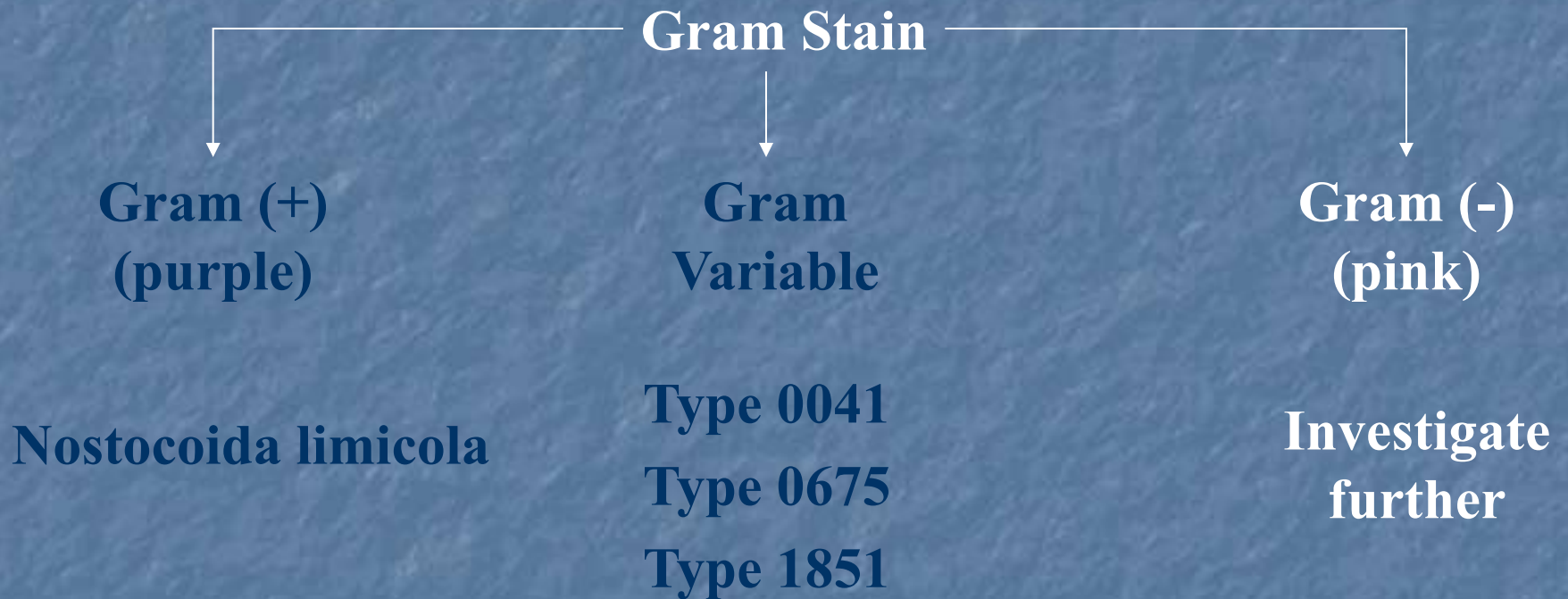
- Type 0041
- Type 0675
- Type 1851
 - All three are Gram Variable
 - All three are favored under the same conditions
 - Low F/M and Low nutrients

Filamentous Bacteria

Bulking Filaments

- Nostocoida limicola
- Sphaerotilus natans
- Haliscomenobacter Hydrossis
- Thiothrix I & II
- Microthrix parvicella
- Beggiatoa
- Type 0041
- Type 0675
- Type 0092
- Type 1851
- Type 1701
- Type 021N
- Type 0914
- Type 0961

Bulking Filaments



Bulking Filaments

Neisser Stain

```
graph TD; A[Neisser Stain] --> B["Neisser (+)  
(bluish)"]; A --> C["Neisser (-)  
(brownish)"]; B --> D["Nostocoida limicola  
Type 0092"]; C --> E["All others"];
```

Neisser (+)
(bluish)

Nostocoida limicola
Type 0092

Neisser (-)
(brownish)

All others

Bulking Filaments



Filamentous Bacteria

Bulking Filaments

- Nostocoida limicola
- Sphaerotilus natans
- Haliscomenobacter Hydrossis
- Thiothrix I & II
- Microthrix parvicella
- Beggiatoa
- Type 0041
- Type 0675
- Type 0092
- Type 1851
- Type 1701
- Type 021N
- Type 0914
- Type 0961

Neisser Positive
Nostocoida Limicola



Neisser Positive

Type 0092



Neisser Positive

Type 0092

- This filament is associated with
 - Low F:M
 - Excess oils and grease
 - Colder temperatures

Filamentous Bacteria

Bulking Filaments

- Nostocoida limicola
- Sphaerotilus natans
- Haliscomenobacter Hydrossis
- Thiothrix I & II
- Microthrix parvicella
- Beggiatoa
- Type 0041
- Type 0675
- Type 0092
- Type 1851
- Type 1701
- Type 021N
- Type 0914
- Type 0961

Bulking Filaments

Neisser Stain

```
graph TD; A[Neisser Stain] --> B["Neisser (+)  
(bluish)"]; A --> C["Neisser (-)  
(brownish)"]; B --> D["Nostocoida limicola  
Type 0092"]; C --> E["All others"];
```

Neisser (+)
(bluish)

Nostocoida limicola
Type 0092

Neisser (-)
(brownish)

All others

Bulking Filaments

Other Characteristics

- Sheath
- Epiphyte (Attached growth)
- Sulfur granules
- Motility

Bulking Filaments

Sheath

- Sheath w/Epiphyte
 - Type 1701
 - Type 0041
 - Type 0675
 - Type 1851
- Sheath w/o Epiphyte
 - Sphaerotilus natans
 - Thiothrix I & II
 - Haliscomenobacter hydrossis

Bulking Filaments

Sheath

- Sheath w/Epiphyte (Attached Growth)
 - Type 1701
 - Type 0041*
 - Type 0675*
 - Type 1851*

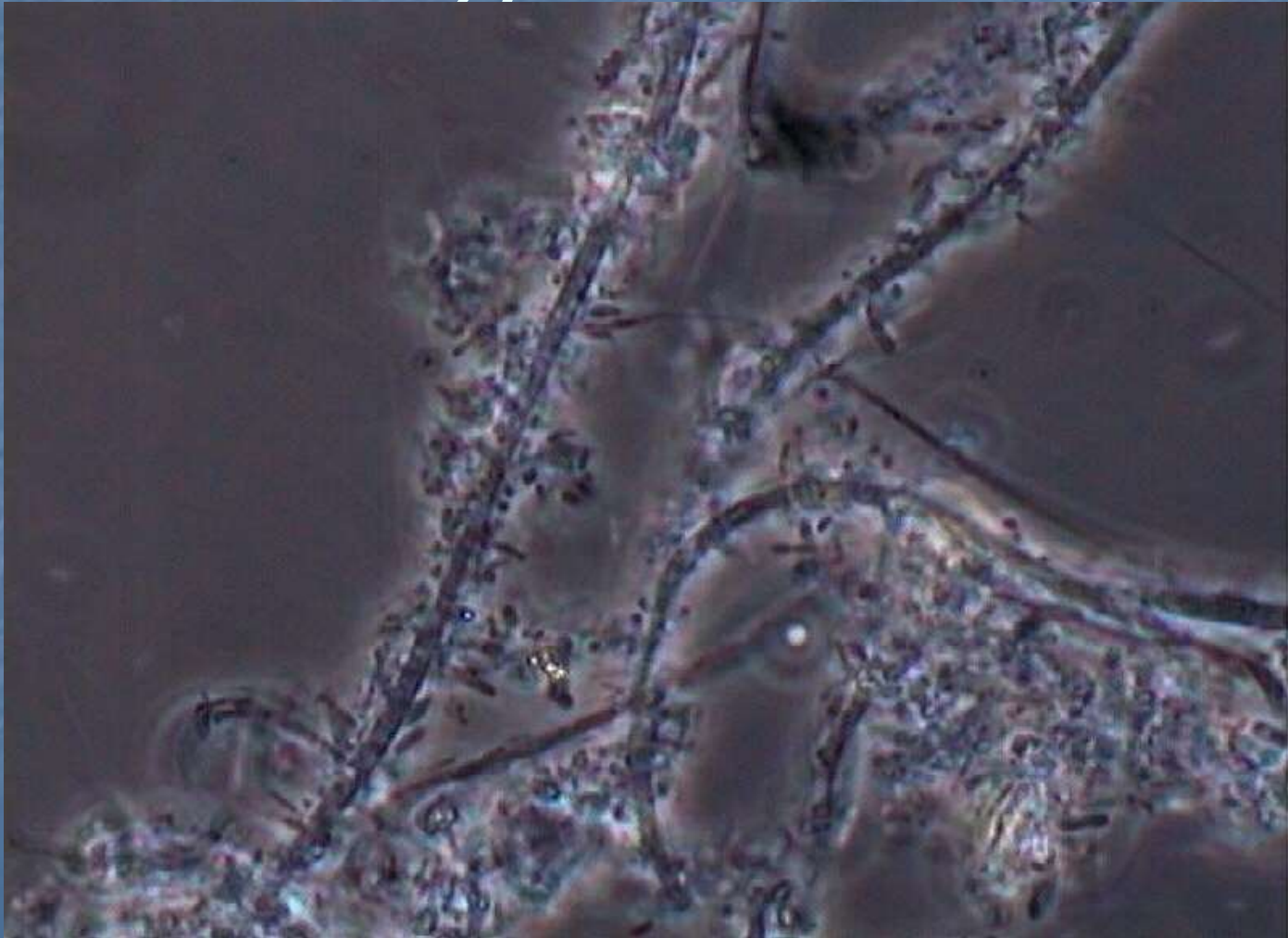
Filamentous Bacteria

Bulking Filaments

- Nostocoida limicola
- Sphaerotilus natans
- Haliscomenobacter Hydrossis
- Thiothrix I & II
- Microthrix parvicella
- Beggiatoa
- Type 0041
- Type 0675
- Type 0092
- Type 1851
- Type 1701
- Type 021N
- Type 0914
- Type 0961

Sheath w/Epiphyte

Type 0041



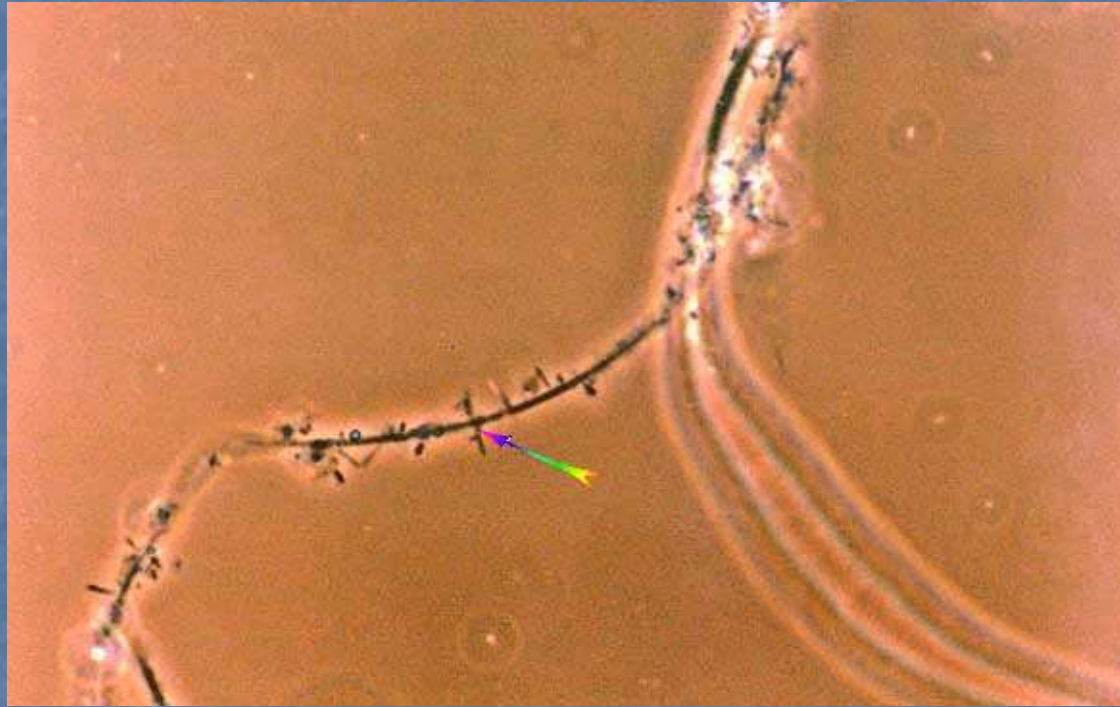
Sheath w/Epiphyte

Type 0675



Sheath w/Epiphyte

Type 1851



Sheath w/Epiphyte

Type 1701



Type 1701



Sheath w/Epiphyte

Type 1701

- This filament is associated with low DO conditions

Filamentous Bacteria

Bulking Filaments

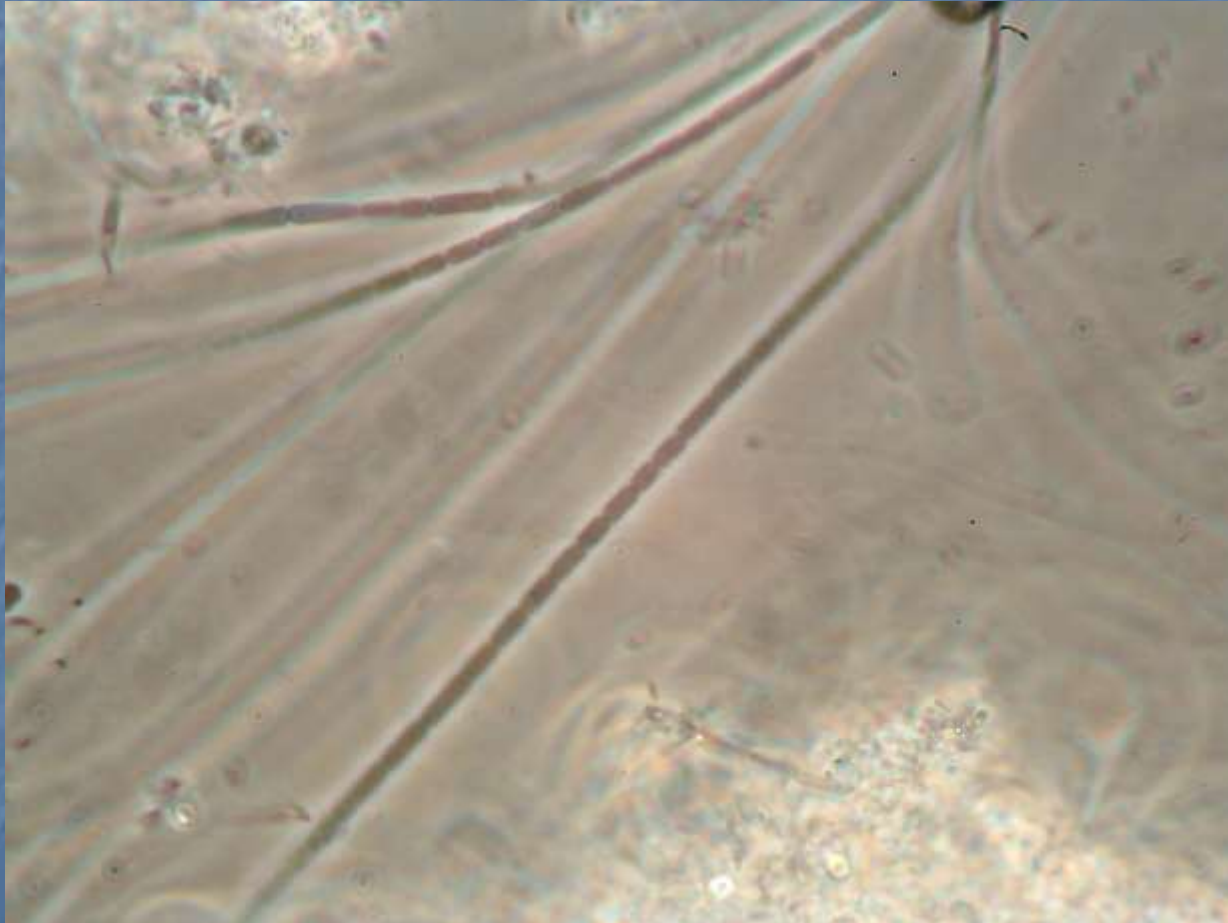
- Nostocoida limicola
- Sphaerotilus natans
- Haliscomenobacter Hydrossis
- Thiothrix I & II
- Microthrix parvicella
- Beggiatoa
- Type 0041
- Type 0675
- Type 0092
- Type 1851
- Type 1701
- Type 021N
- Type 0914
- Type 0961

Sheath w/o Epiphyte

- Sheath w/o Epiphyte (Attached growth)
 - *Sphaerotilus natans*
 - *Thiothrix* I & II
 - *Haliscomenobacter hydrossis*

Sheath w/o Epiphyte

Sphaerotilus natans



Sheath w/o Epiphyte

Sphaerotilus natans



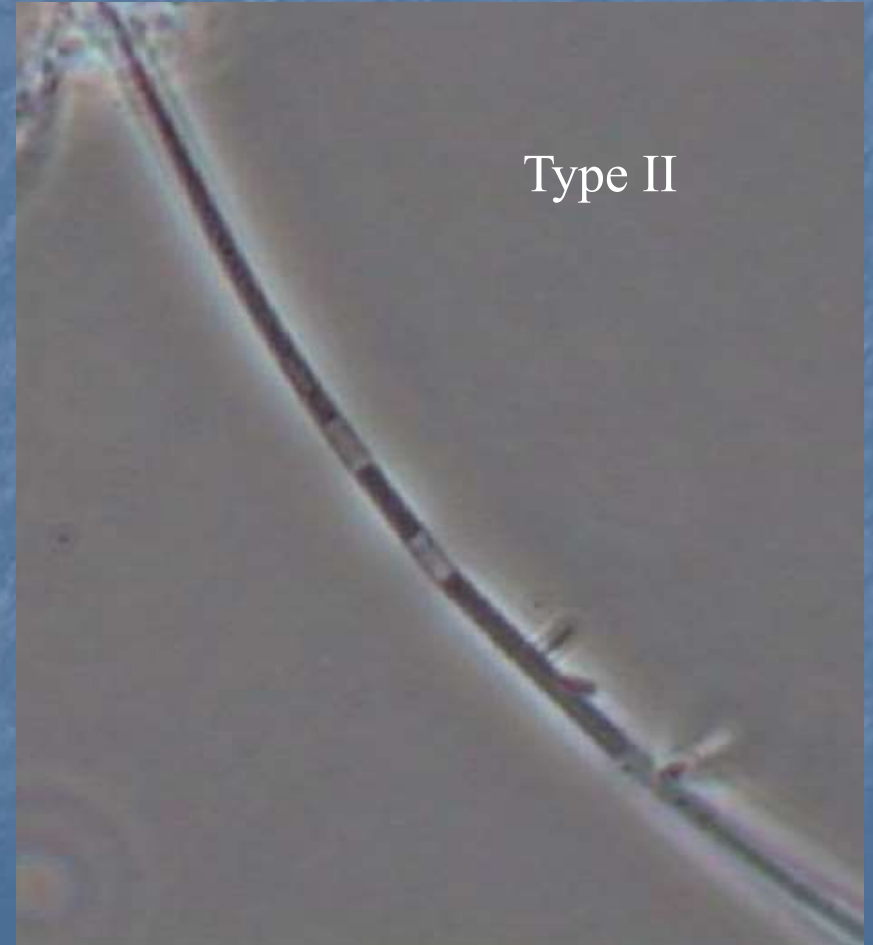
Sheath w/o Epiphyte

Sphaerotilus natans

- *S. natan* also exhibits false branching
- Associated with conditions where there is inadequate DO for the applied organic loading

Sheath w/o Epiphyte

Thiothrix I & II



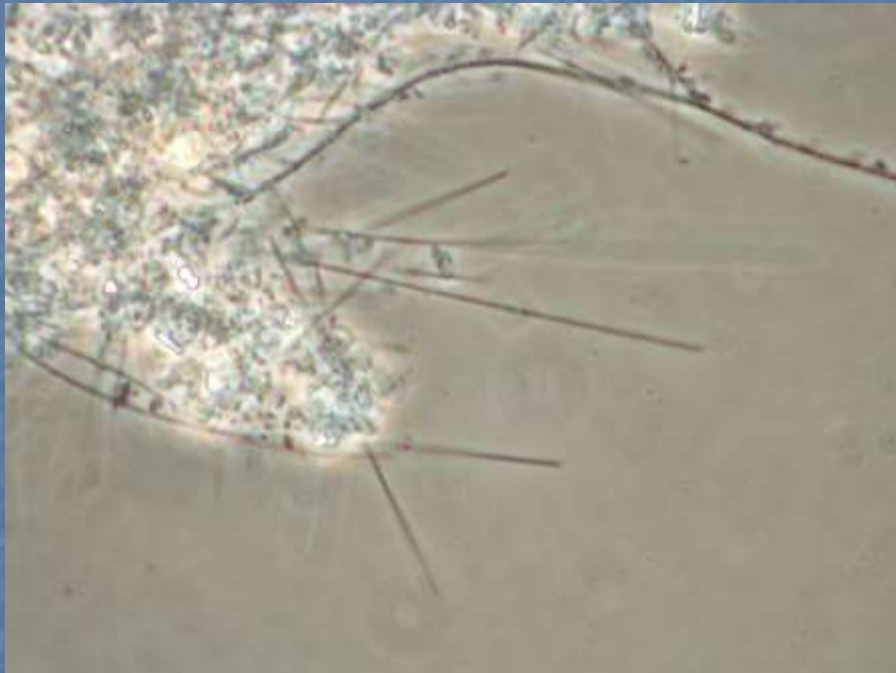
Sheath w/o Epiphyte

Thiothrix I & II

- This filament is associated with
 - The presence of reduced sulfur compounds (septic wastes)
 - Wastes deficient in nitrogen
 - The presence of organic acids

Sheath w/o Epiphyte

H. Hydrossis



Sheath w/o Epiphyte

H. Hydrossis

- This filament is associated with low DO conditions

Filamentous Bacteria

Bulking Filaments

- Nostocoida limicola
- Sphaerotilus natans
- Haliscomenobacter Hydrossis
- Thiothrix I & II
- Microthrix parvicella
- Beggiatoa
- Type 0041
- Type 0675
- Type 0092
- Type 1851
- Type 1701
- Type 021N
- Type 0914
- Type 0961

Bulking Filaments

Sulfur Granules

- Type 021N
- Thiothrix I & II*
- Type 0914
- Beggiatoa

Sulfur Granules

Thiothrix I



Sulfur Granules

Thiothrix I



Sulfur Granules

Thiothrix II



Bulking Filaments

Sulfur Granules

- These filaments are all associated with
 - The presence of reduced sulfur compounds (septic wastes)
 - Wastes deficient in nitrogen
 - The presence of organic acids

Sulfur Granules

Type 021N



Sulfur Granules

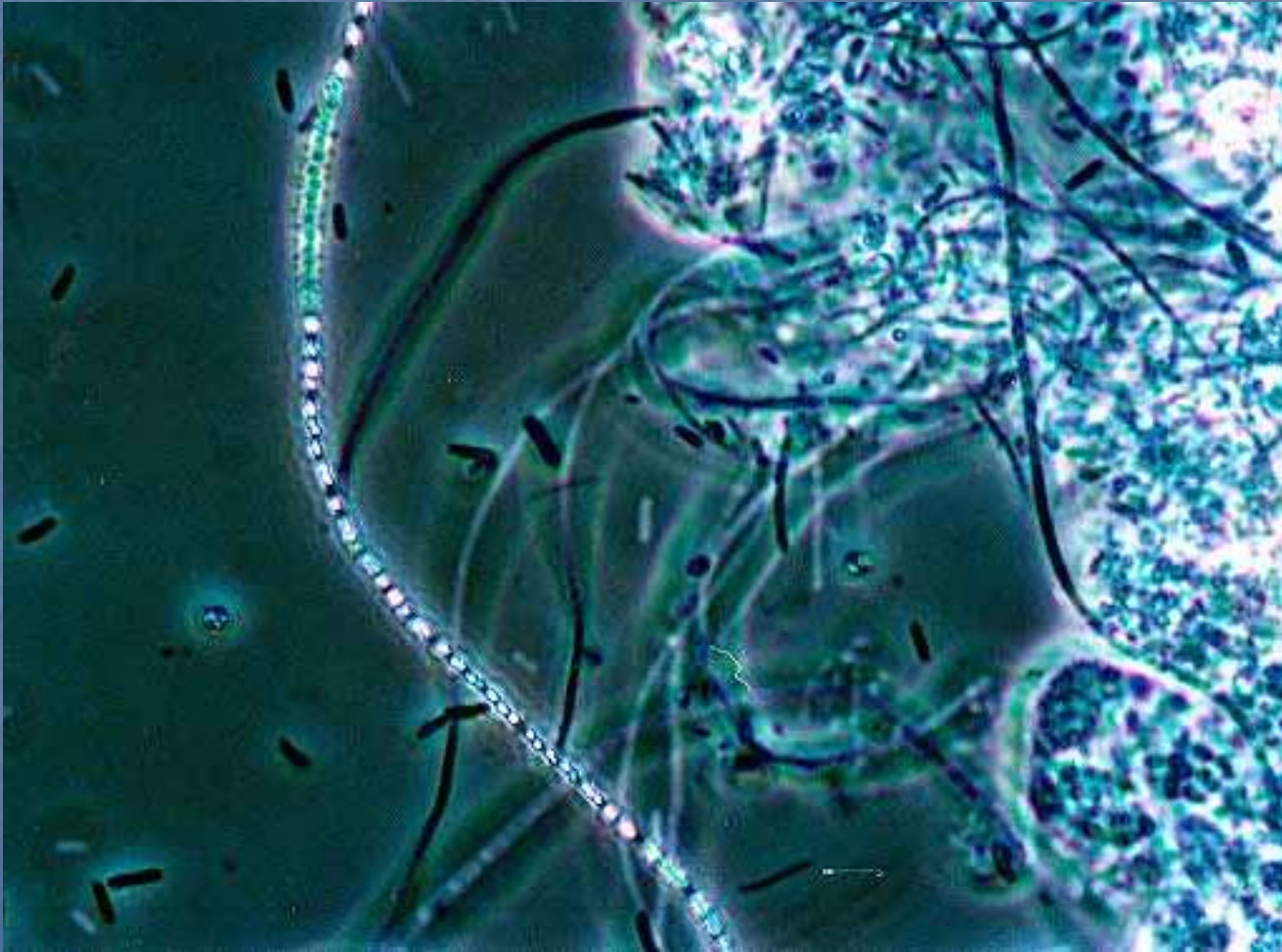
Type 021N



Stacked (hockey puck)
cells

Sulfur Granules

Type 0914



Sulfur Granules

Beqgiatoa



Filamentous Bacteria

Bulking Filaments

- Nostocoida limicola
- Sphaerotilus natans
- Haliscomenobacter Hydrossis
- Thiothrix I & II
- Microthrix parvicella
- Beggiatoa
- Type 0041
- Type 0675
- Type 0092
- Type 1851
- Type 1701
- Type 021N
- Type 0914
- Type 0961

Bulking Filaments

Motility

- Beggiatoa*

Bulking Filaments

Motility

- Beggiatoa is the only filament that is motile
- The presence of this filament is associated with
 - Organic overloading
 - Septic conditions
 - Most often found in trickling filters and RBCs

Bulking Filaments

Beggiatoa



Filamentous Bacteria

Bulking Filaments

- Nostocoida limicola
- Sphaerotilus natans
- Haliscomenobacter Hydrossis
- Thiothrix I & II
- Microthrix parvicella
- Beggiatoa
- Type 0041
- Type 0675
- Type 0092
- Type 1851
- Type 1701
- Type 021N
- Type 0914
- Type 0961

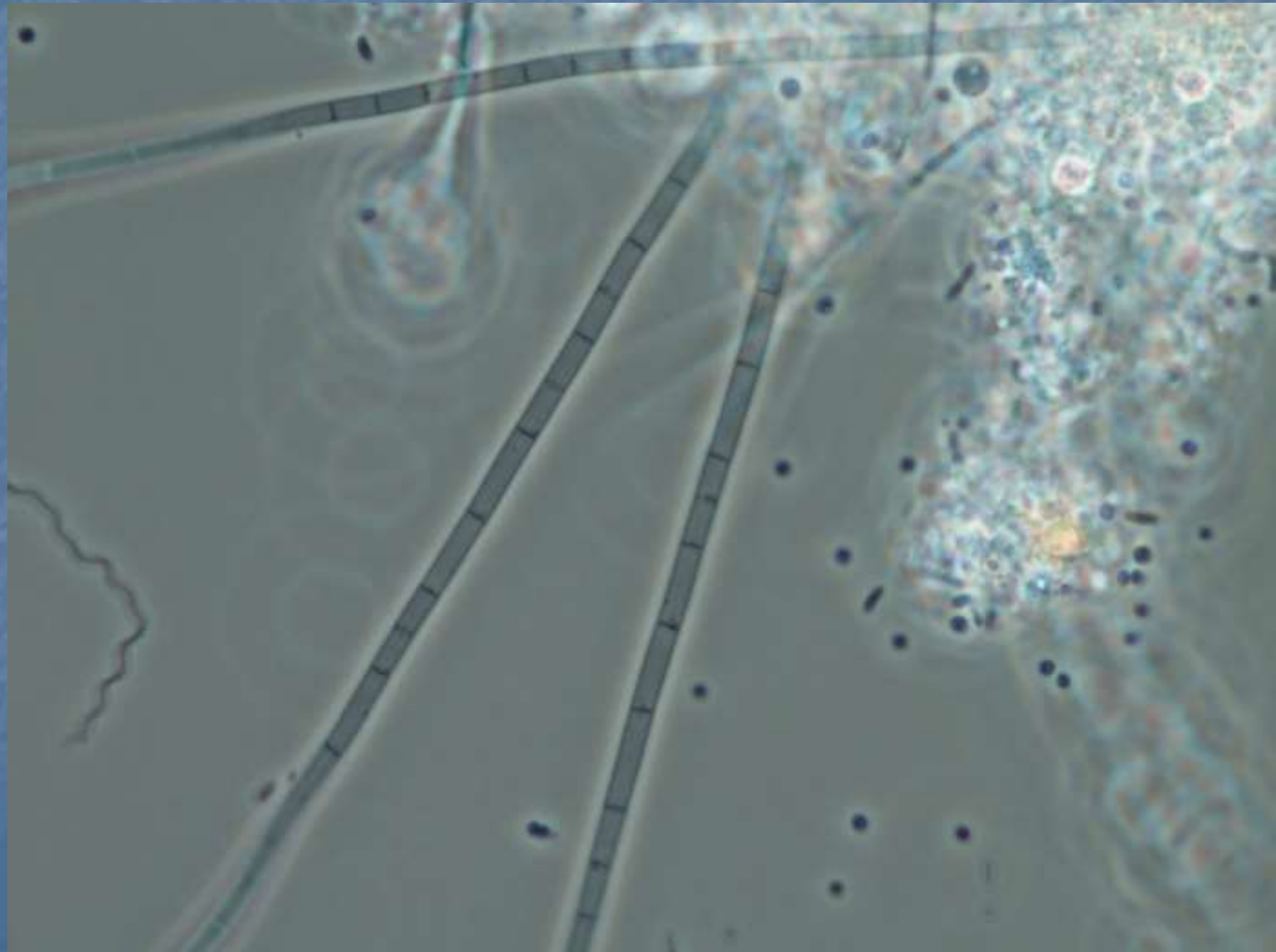
Bulking Filaments

Type 0961

- Not Gram +
- Not Neisser +
- No sheath
- No attached growth
- No sulfur granules
- Not motile

Bulking Filaments

Type 0961



Bulking Filaments

Type 0961



Bulking Filaments

Type 0961

- Low F:M
- Low soluble BOD

Filamentous Bacteria

Foaming Filaments

- All foaming filaments are associated with:
 - Excess greases, oils and fats
 - Low F:M
 - Hydrophobic cell walls

Filamentous Bacteria

Foaming Filaments

- Collect a sample of the foam
- Make a smear
- Gram stain

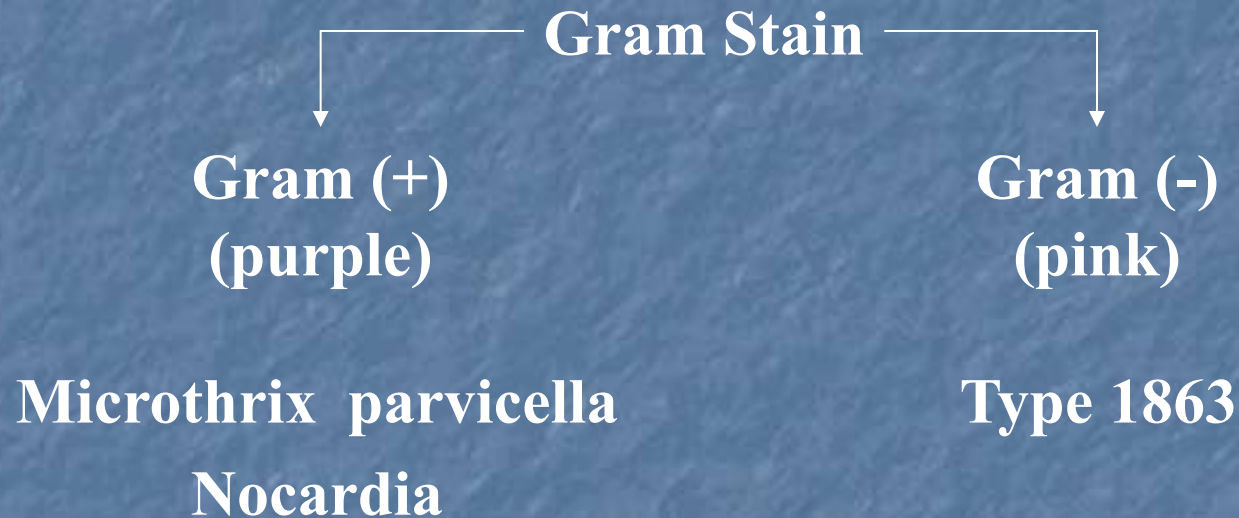
Filamentous Bacteria

Foaming Filaments

- Type 1863
- *Microthrix parvicella*
- *Nocardia*

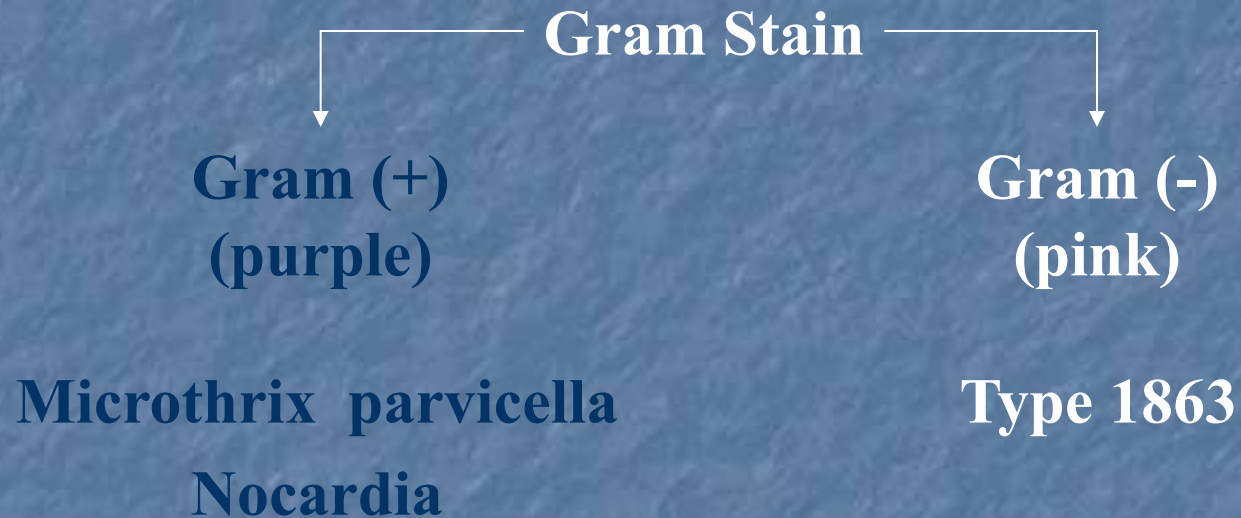
Filamentous Bacteria

Foaming Filaments



Filamentous Bacteria

Foaming Filaments



Foaming Filaments *Type* *1863*



Foaming Filaments *Type* *1863*



Foaming Filaments *Type* *1863*

- Type 1863 is associated with
 - Excess grease and fat
 - Low F:M
 - Decline in aeration basin pH

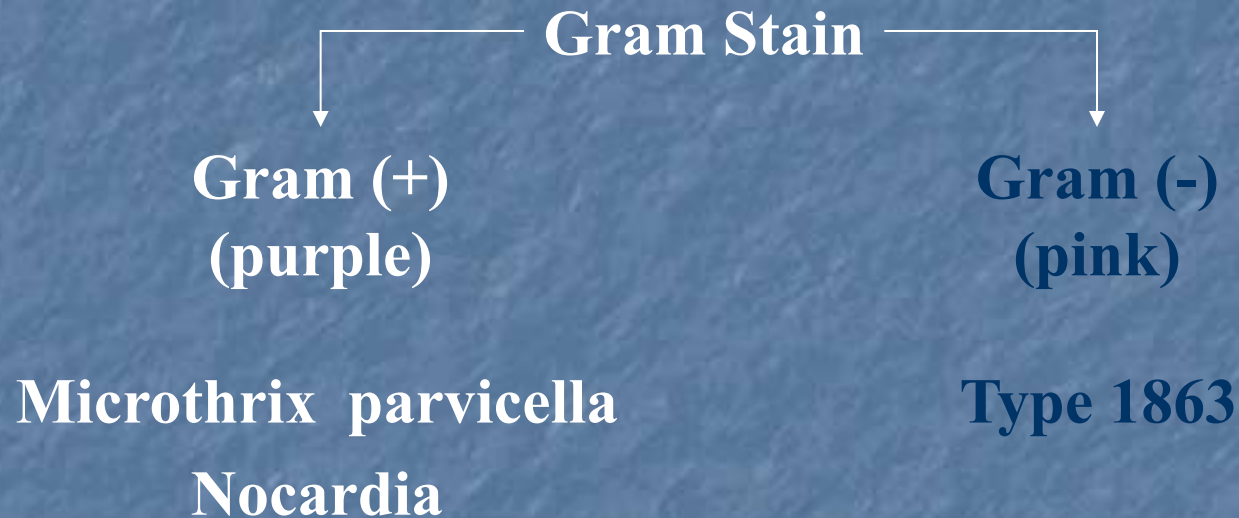
Filamentous Bacteria

Foaming Filaments

- Type 1863
- *Microthrix parvicella*
- *Nocardia*

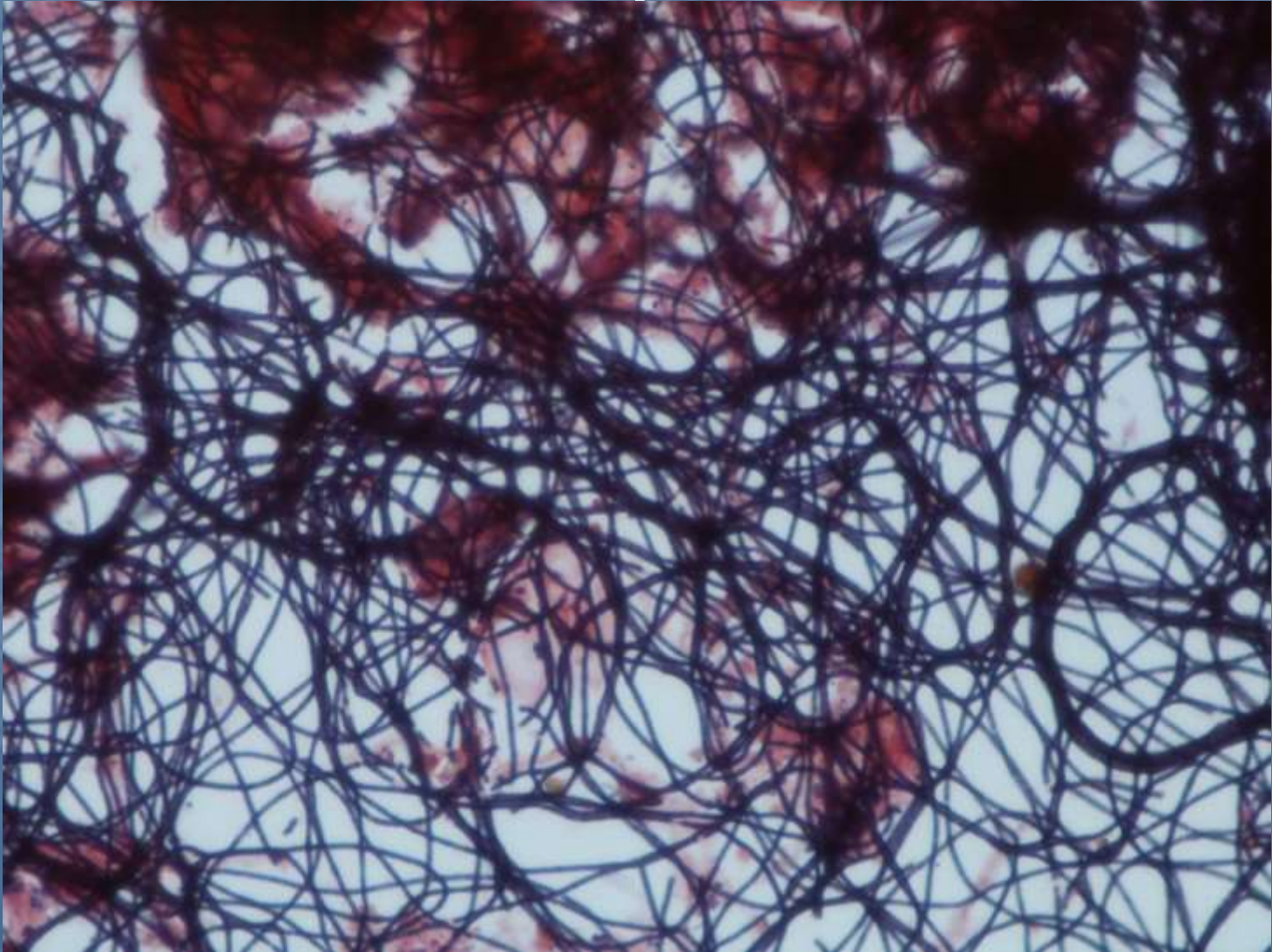
Filamentous Bacteria

Foaming Filaments



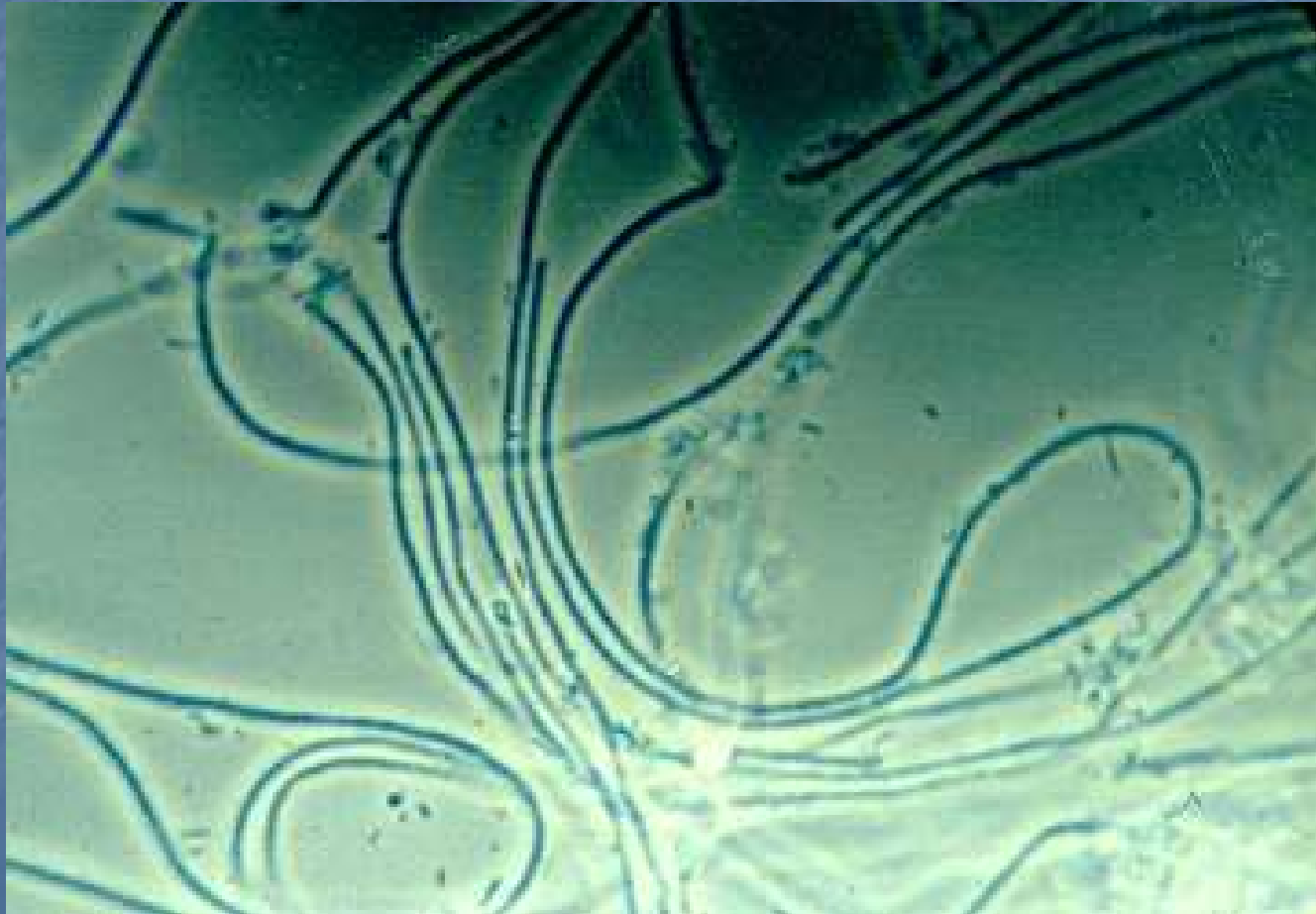
Filamentous Bacteria

Microthrix parvicella



Filamentous Bacteria

Microthrix parvicella



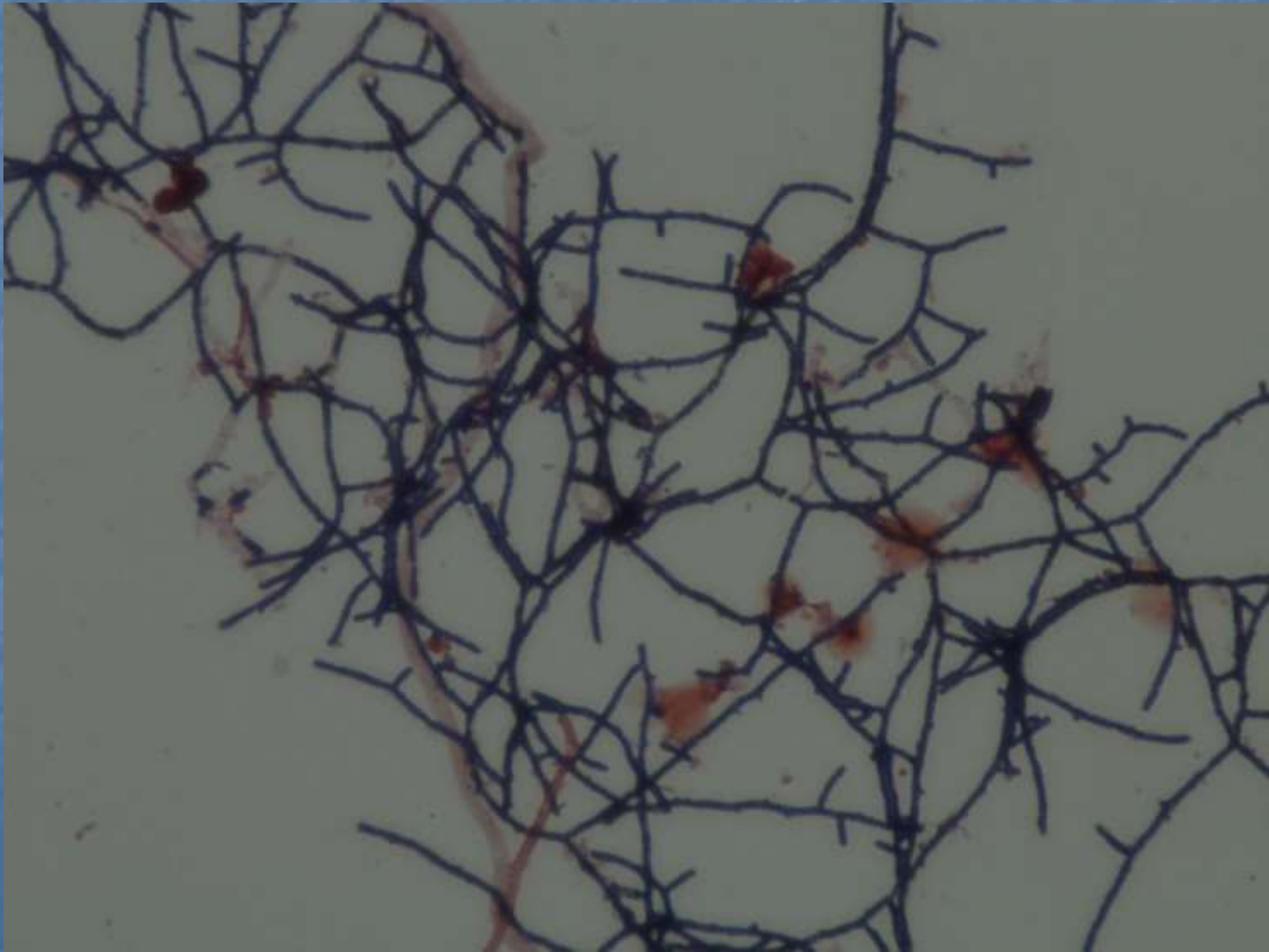
Filamentous Bacteria

Microthrix parvicella

- *Microthrix parvicella* is associated with
 - Animal and vegetable greases and fats
 - Low F:M
 - Colder temperatures

Filamentous Bacteria

Nocardia



Filamentous Bacteria

Nocardia

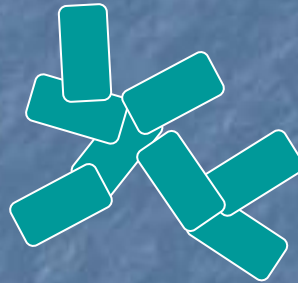
- This filament is strongly associated with
 - Long MCRTs (sludge age)
 - Greases, oils, fats, petroleum, rubber
 - Warmer temperatures

Filamentous Bacteria

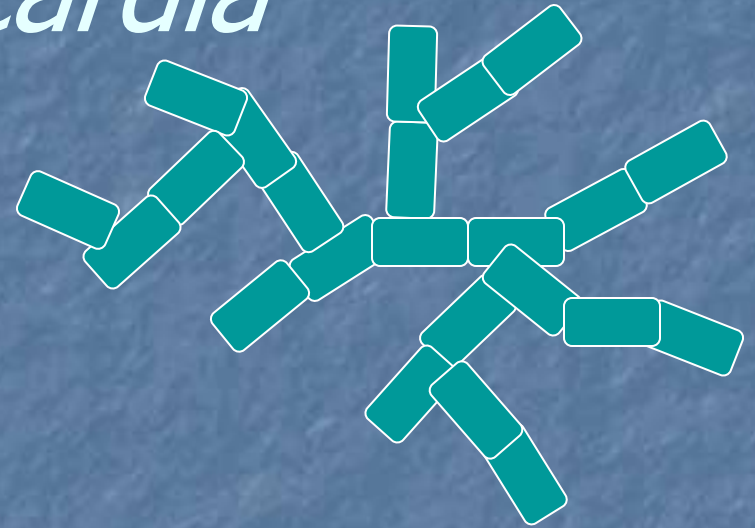
About Nocardia



No foaming



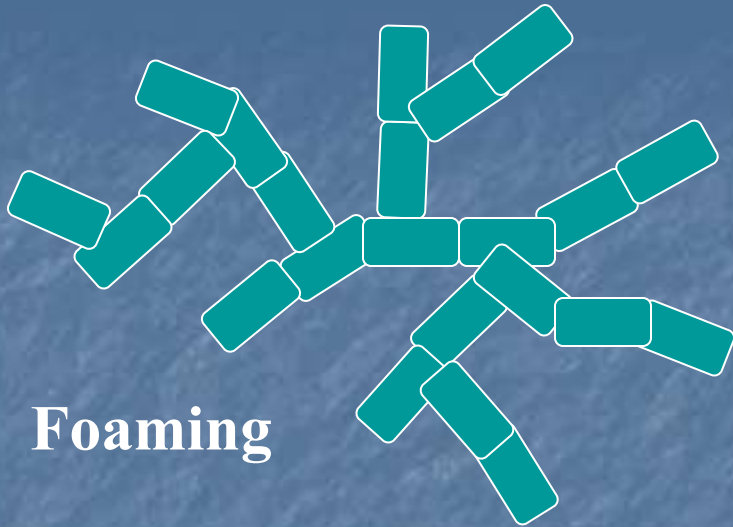
Very little foaming



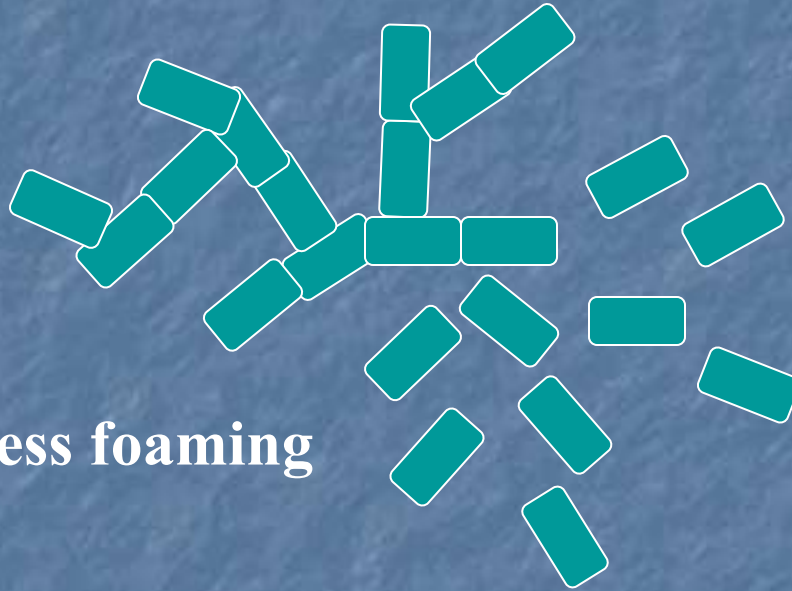
Foaming

Filamentous Bacteria

About Nocardia



Foaming



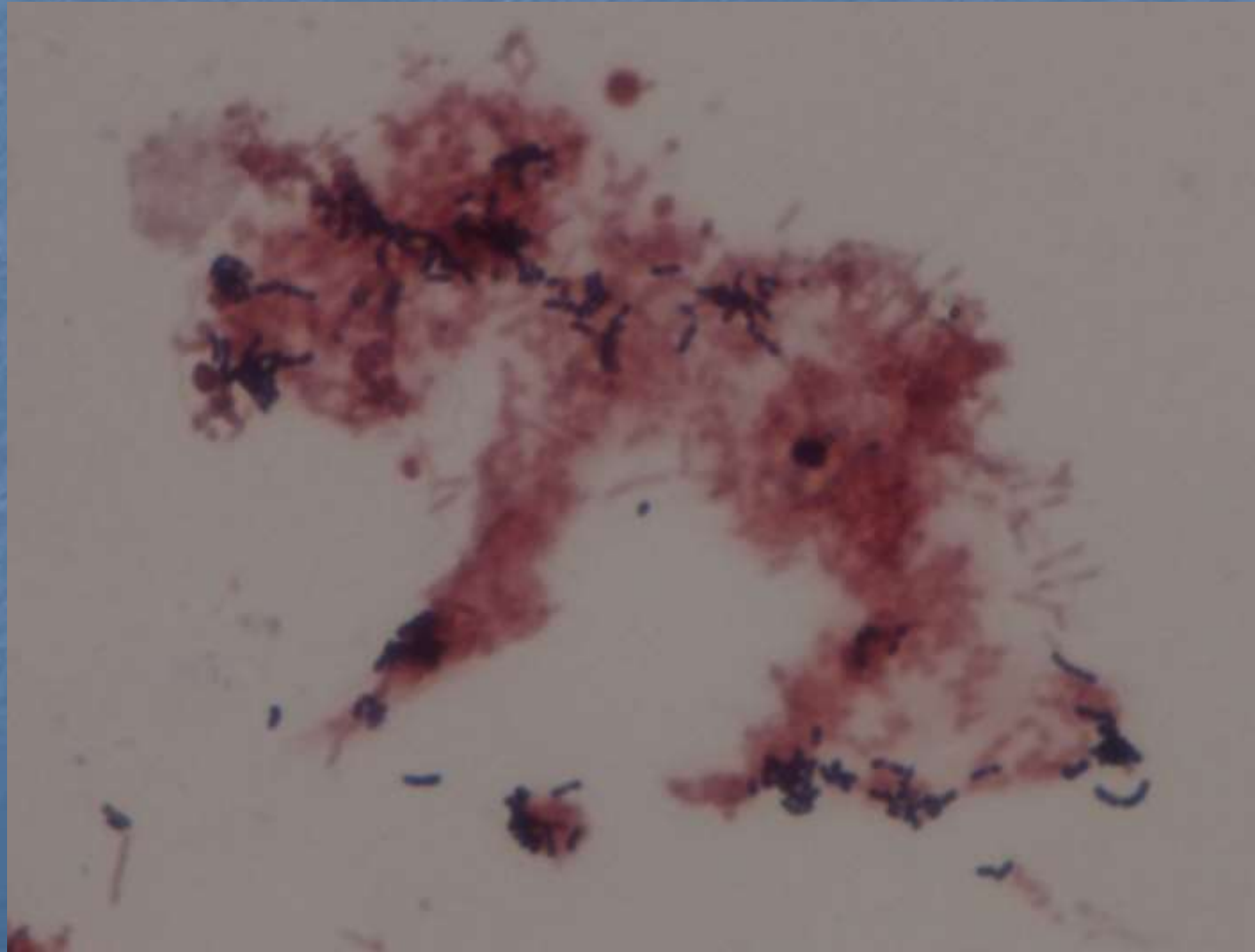
Less foaming



No foaming

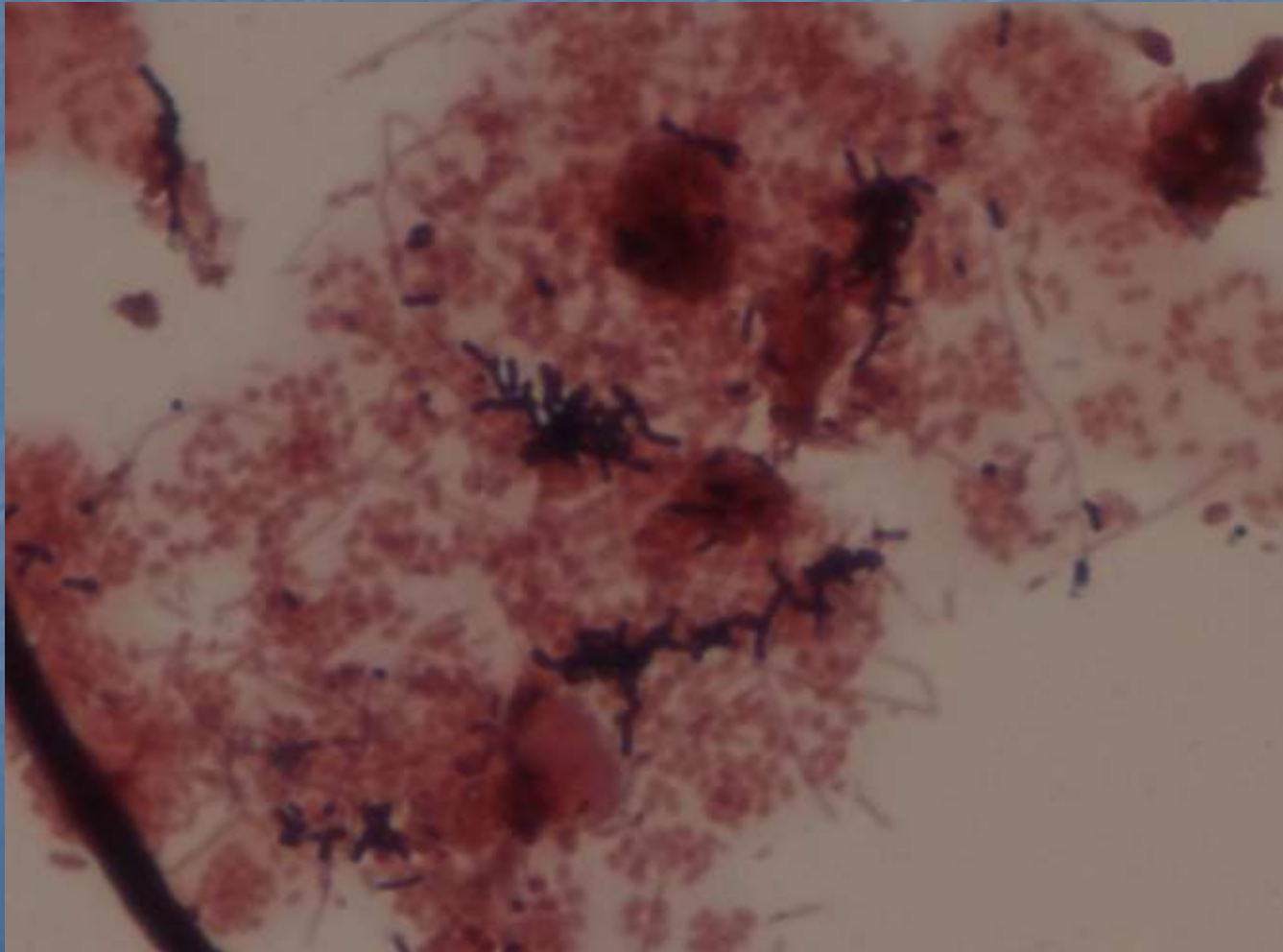
Nocardia

Early Stage Nocardia



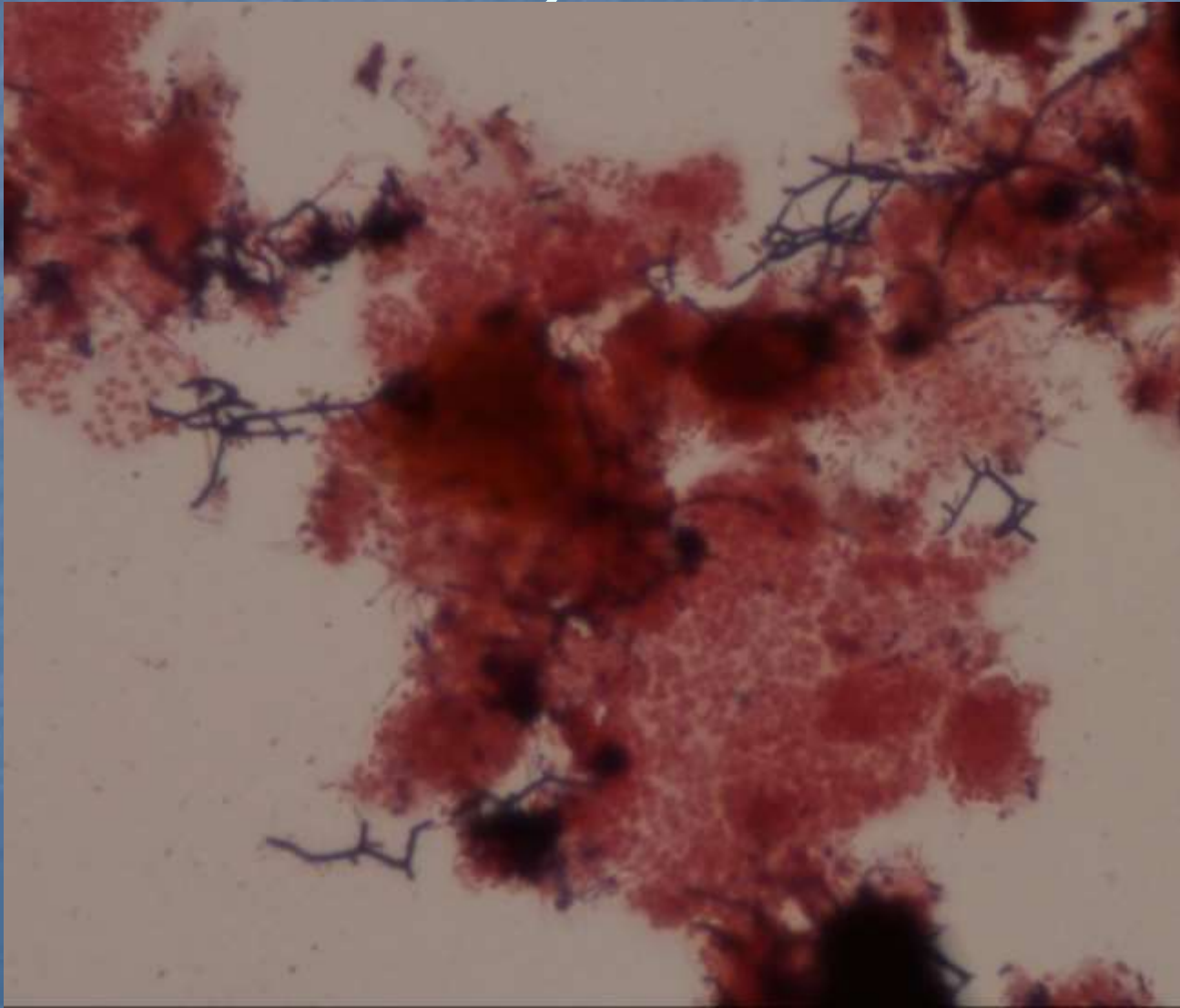
Nocardia

Early Stage Nocardia



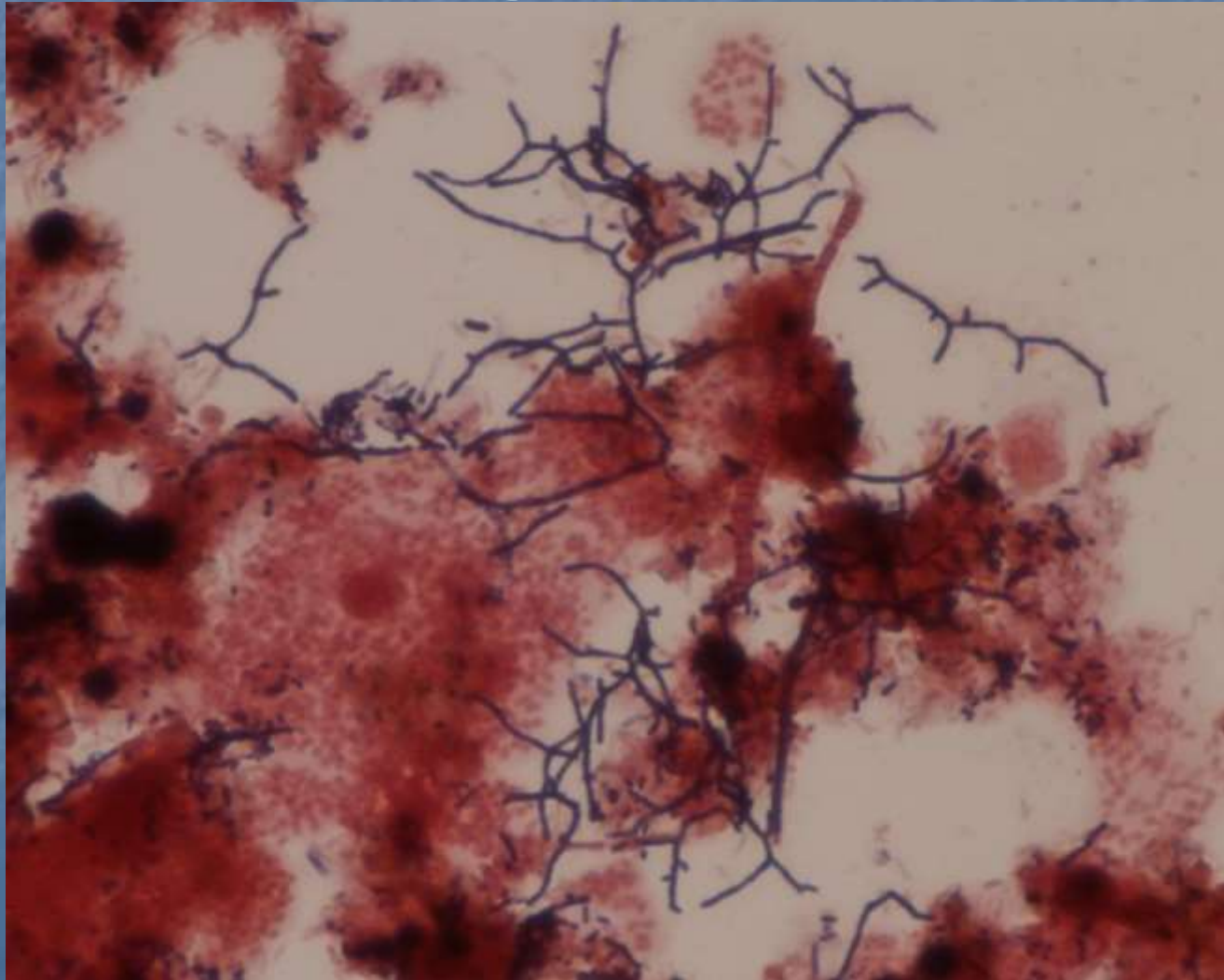
Nocardia

Mid - Stage Nocardia



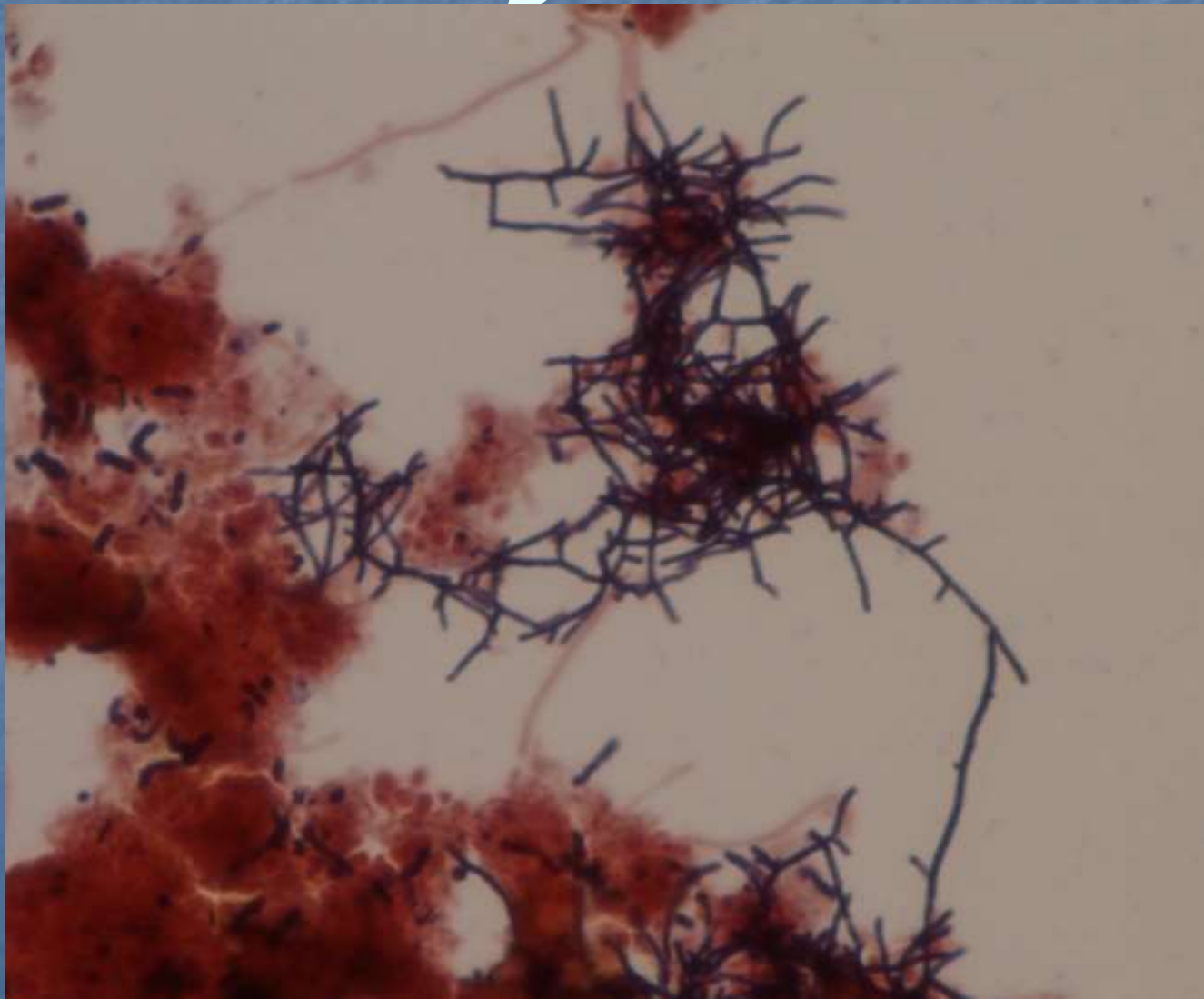
Nocardia

Mid - Stage Nocardia



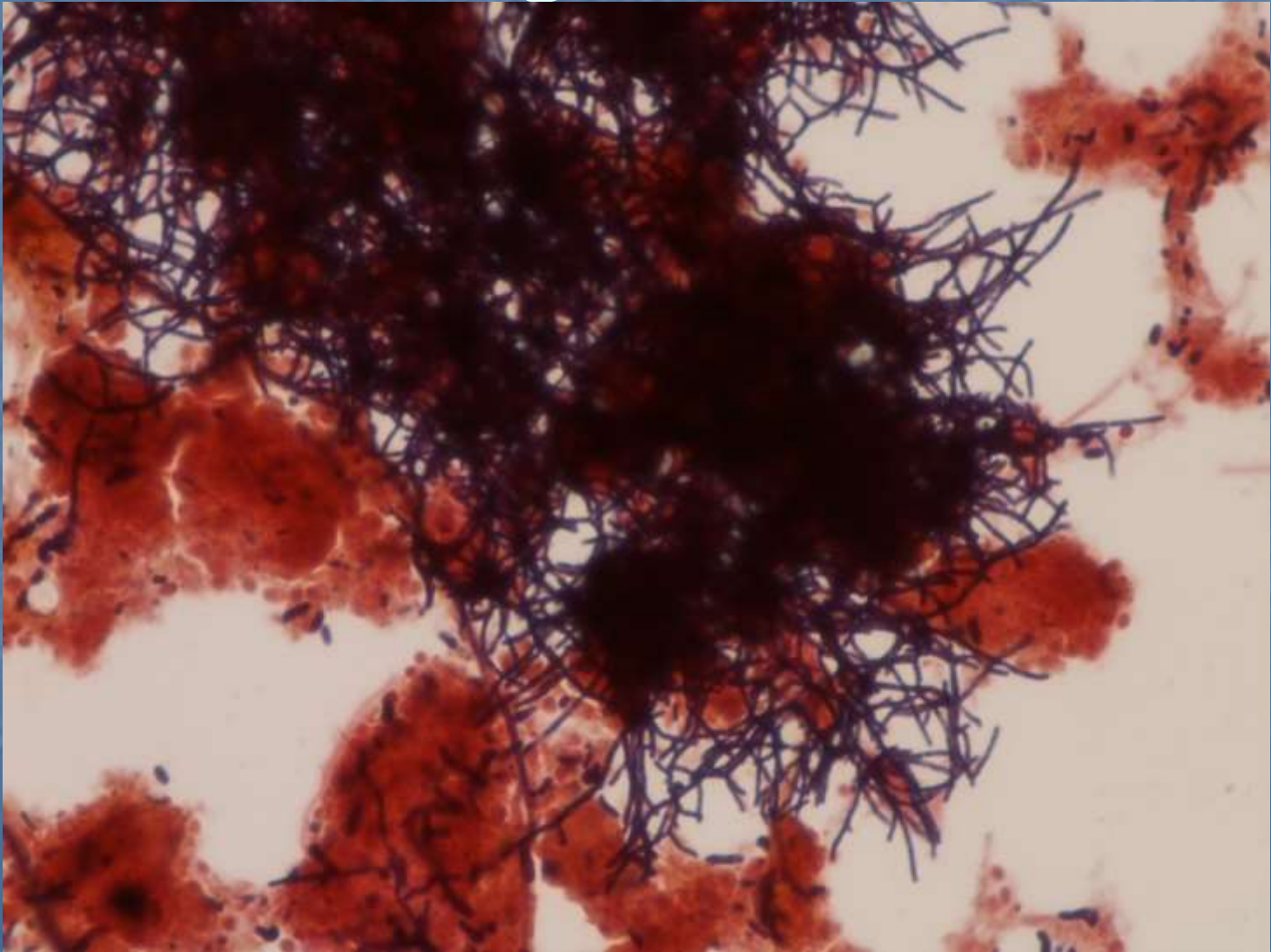
Nocardia

Late Stage Nocardia



Nocardia

Late Stage Nocardia



Nocardia

Adult Stage Nocardia



Filamentous Bacteria

Foaming Filaments

- Type 1863
- *Microthrix parvicella*
- *Nocardia*

Foaming Filaments

Neisser Stain

```
graph TD; A[Neisser Stain] --> B["Neisser (+)  
(bluish)"]; A --> C["Neisser (+)  
Granules"]; A --> D["Neisser (-)  
(brownish)"]; B --- E["Nostocoida limicola  
Type 0092"]; C --- F["Microthrix  
Nocardia"]; D --- G["All others"];
```

Neisser (+)
(bluish)

Nostocoida limicola
Type 0092

Neisser (+)
Granules

Microthrix
Nocardia

Neisser (-)
(brownish)

All others

Neisser Positive Granules

Microthrix



Neisser Positive Granules

Nocardia



Filament Control

- For low F:M or low food
 - Add more food or get rid of some bugs
- For low DO
 - Add more oxygen

Filament Control

- Excess greases, oils and fats
 - Grease control
- For a long MCRT or Sludge age
 - Shorten it
- Sulfur granules
 - Pre-aeration, add oxygen

Most Important

- Keep a process chart of treatment system parameters
- Measure the parameters routinely and consistently
- Measure the parameters when the treatment system is working properly