Environmental Biotechnology

CE421/521

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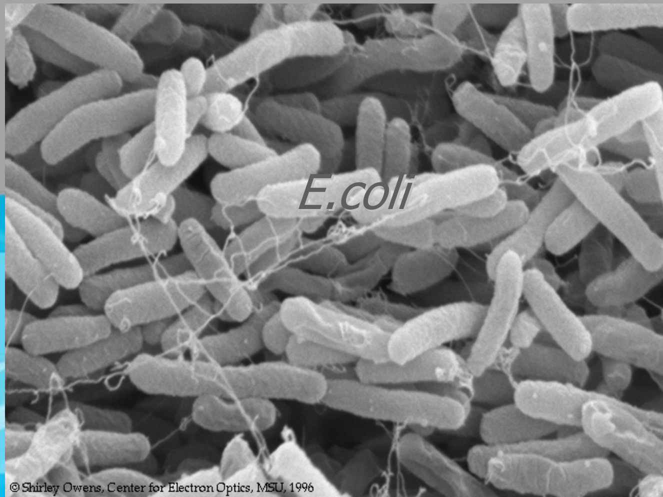
INDICATOR MICROORGANISMS

- *Need for indicator organisms*
  - *Not possible to test for every pathogen*
  - *Use indicator that will be easy to test routinely*
  - *Early warning if there is a problem*
  - *Used in regulations*
    - *Water treatment and distribution*
    - *Wastewater treatment discharges (NPDES permit)*
    - *When to close beaches and lakes for recreational uses*
      - *Beaches closed when FC is over 300 colony forming units per 100 mL or when monthly average is over 140 CFU per 100 mL*

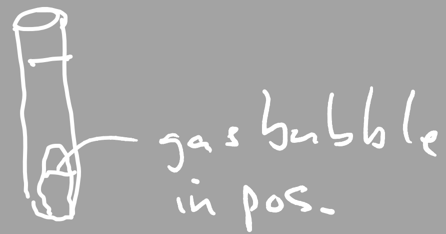
INDICATOR MICROORGANISMS

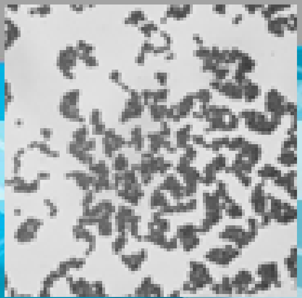
- Use of indicator organisms dates back to 1914 when U.S. Public Health Service adopted the coliform test as an indication of fecal contamination
- Ideal indicator should have the following characteristics:
  1. Found in intestines of warm blooded animals
  2. Should be present when pathogen are present and absent when pathogens are absent
  3. Present in greater number than pathogens
  4. As resistant as (or more resistant than) pathogens
  5. It shouldn't multiply in the environment
  6. Easily detectable by rapid, inexpensive method
  7. Non-pathogenic itself

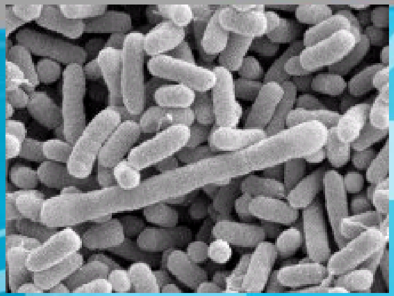
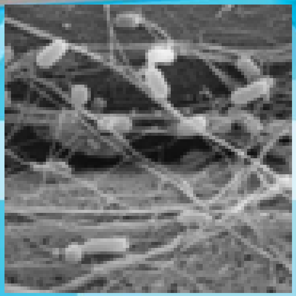


Total Coliforms

- Characteristics:
  - Aerobic and f_acultative_____ anaerobic organisms
  - gram n_egative_____
  - non s_pore_____ forming
  - r_od_____ shaped
  - ferment l_actose_____ within 48 h at 35°C as evidenced by gas production
  - includes *E. coli*, *Enterobacter*, *Kleibsiella*, and *Citrobacter*
  - high levels in human and animal feces _10^9_____ per capita per day





Fecal Coliforms

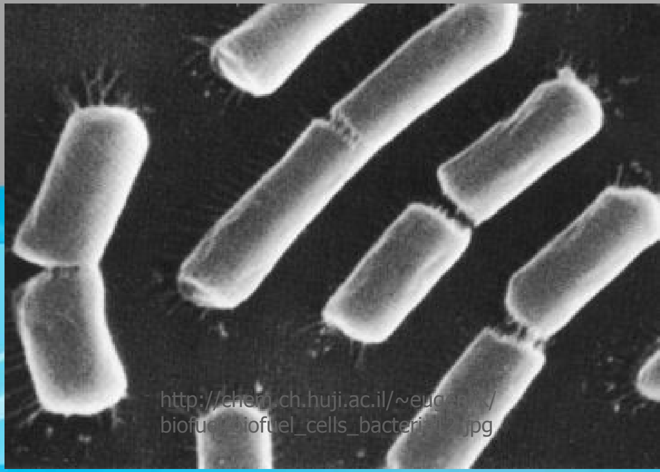
- all coliforms that can ferment lactose at 44.5°C as evidenced by g<u>as</u>_______ production
- includes groups such as *E. coli* and *Kleibsiella*
- p<u>resence</u>_______________ is an indication of human and animal contamination
- human and animal contamination cannot be d<u>ifferentiated</u>_______________
- s<u>urvival</u>__________________ pattern is similar to bacterial pathogens
- much l<u>ess</u>______ resistant to disinfection than protozoan pathogens

Fecal Streptococci

- includes groups such as *Streptococcus faecalis, S. bovis, S. equinus,* and *Kleibsiella*
- inhabit intestines of warm blooded animals and humans
- Historically fecal coliform/fecal strep ratio serves as useful indicator of origin of contamination
  - ratios greater than 4 indicate human origin
  - ratios less than 0.7 indicate animal contamination
  - In-between ratios indicate a mixture of human and animal contamination
  - Currently there are probably better indicators of human contamination (pharmaceuticals and personal care products) → micronutrients

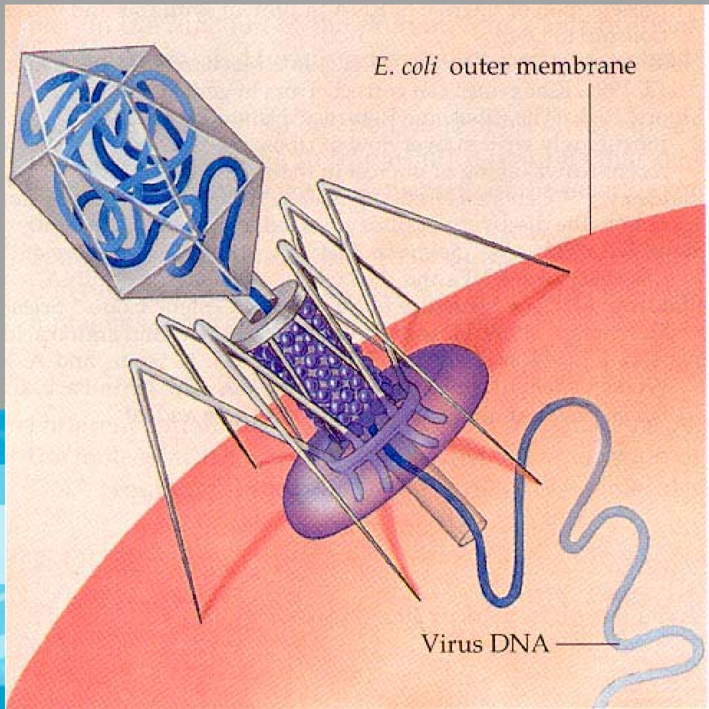
Clostridium perfringens

- Anaerobe
- forms spores____________ that are resistant to disinfection and environmental stress
- possibly too_____ resistant to be useful as an indicator
- good for tracking contamination in marine__________ environments



Bacteriophages

- Similar to enteric viruses and found in higher numbers
- Suggested as water quality indicators in estuaries, seawater, recreational waters, and drinking water
- Coliphages exhibit best correlation to enteric viruses



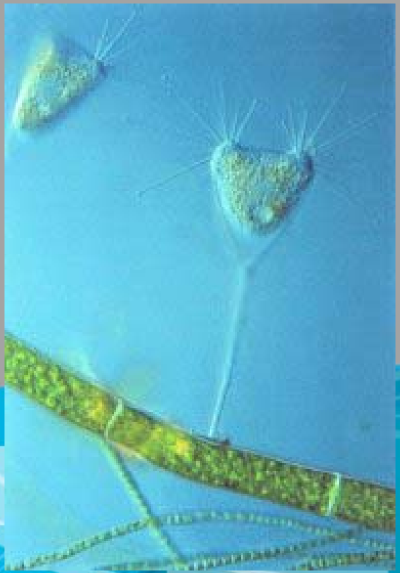
Heterotrophic Plate Count (HPC)

- Measure of aerobic and facultative anaerobic bacteria that derive their carbon and energy from organic compounds
- No known effects of high HPC on human health
- HPC in drinking water ranges from less than 1 CFU/mL to more than 10^4 CFU/mL
- Good indicator of pathogens in reclaimed wastewater

BULKING and FOAMING

- *Sludge settling can be the most important operational problem in an activated sludge plant*

Ciliates



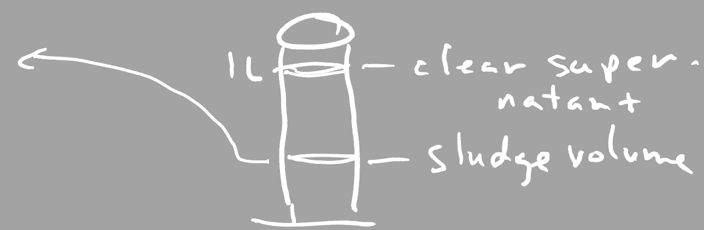
Nematodes

Rotifers

FILAMENTOUS BULKING

- *Measurement of sludge settleability*
- *Sludge v*olume *index* SVI
- *Measure of s*ettling *characteristics*
- *Measured in a g*raduated *cylinder after 30 min of settling*

$\frac{mL}{g}$



- *Units of mL/g*
- *A d*esirable *SVI is in the range of 75-150*

Bulking and Foaming

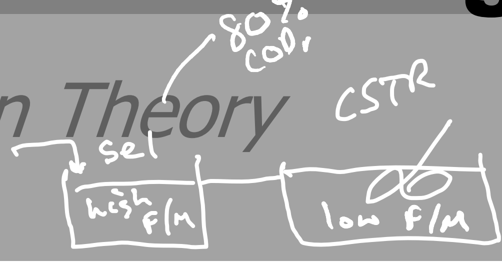
TABLE 9.1. Causes and Effects of Activated Sludge Separation Problems

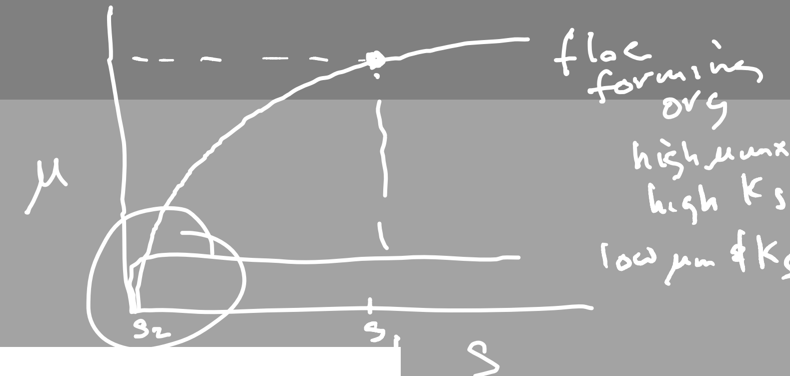
Name of Problem	Cause of Problem	Effect of Problem
Dispersed growth	Microorganisms do not form flocs but are dispersed, forming only small clumps or single sells.	Turbid effluent. No zone settling of sludge.
Slime (jelly); Viscous bulking; (also possibly has been referred to as nonfilamentous resulting in bulking)	Microorganisms are present in large amounts of extracellular slime.	Reduced settling and compaction rates. Virtually no solids separation, in severe cases in overflow sludge blanket from secondary clarifier.
Pin-floc (or pinpoint floc)	Small, compact, weak, roughly speherical flocs are formed, the larger of which settle rapidly. Smaller aggregates settle slowly.	Low sludge volume index (SVI) and a cloudy, turbid effluent.
Bulking	Filamentous organisms extend from flocs into the bulk solution and interfere with compaction and settling of activated sludge.	High SVI—very clear supernatant.
Rising sludge (blanket rising)	Denitrification in secondary clarifier releases poorly soluble N_2 gas, which attaches to activated sludge flocs and floats them to the secondary clarifier surface.	A scum of activated sludge forms on the surface of secondary clarifier.
Foaming/scum formation	Caused by (1) nondegradable surfactants and by (2) by the presence of *Nocardia* sp. and sometimes by (3) the presence *Microthrix parvicella.*	Foams float large amounts activated sludge solids to surface of treatment units. Foam accumulate and putrefy. Solids can overflow into secondary effluent or overflow tank free-board onto walkways.

Adapted from Jenkins et al. (1984)

Filamentous Bulking

- *Kinetic Selection Theory (Chodoba et al.*



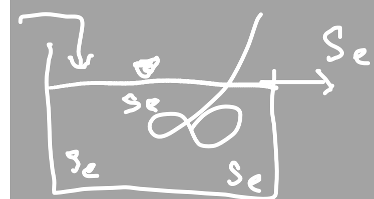


FILAMENTOUS BULKING

TABLE 9.2. Comparison of Physiological Characteristics of Floc-Formers and Filamentous Organisms

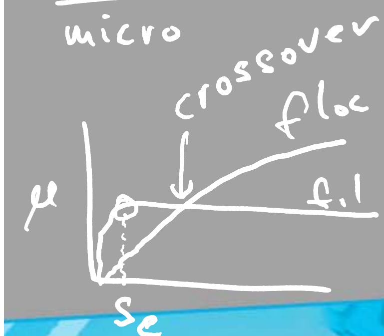
Characteristic	Bacteria	
	Floc-Formers	Filamentous
Maximum substrate uptake rate	High	Low
Maximum specific growth rate	High	Low
Endogenous decay rate	High	Low
Decrease in specific growth rate from low substrate concentration	Significant	Moderate
Resistance to starvation	Low	High
Decrease in specific growth rate from low DO	Significant	Moderate
Potential to sorb organics when excess is available	High	Low
Ability to use nitrate as an electron acceptor	Yes	No
Exhibits luxury uptake of phosphorus	Yes	No

From Sykes (1989).



low F/M

food / micro



Types of Filaments

- filament shape______
- size and shape within filament
- branching______
- motility (e.g. *Beggiatoa* move by gliding______)
- presence of sheath______
- presence of epiphytic bacteria______ on filament surface
- filament size and diameter______
- presence of granules______

Isolation of Filaments

- microscopic____________ analysis
- fluorescent-antibody____________ techniques
- RNA chemotaxonomy (gene________ probes________)

Predominant Filaments

TABLE 9.3. Filamentous Organisms Predominant in U.S. Bulking Activated Sludges

Rank	Filamentous Organism	Percentage of Treatment Plants with Bulking Sludge Where Filament was Observed to Be Dominant[a]
1	*Nocardia* spp.	31
2	Type 1701	29
3	Type 021N	19
4	Type 0041	16
5	*Thiothrix* spp.	12
6	*Sphaerotilus natans*	12
7	*Microthrix parvicella*	10
8	Type 0092	9
9	Haliscomenobacter hydrossis	9
10	Type 0675	7
11	Type 0803	6
12	*Nostocoida limicola*	6
13	Type 1851	6
14	Type 0961	4
15	Type 0581	3
16	*Beggiatoa* spp.	<1
17	Fungi	<1
18	Type 0914	<1
	All others	<1

[a]Percentage of 525 samples from 270 treatment plants with bulking problems.
From Jenkins and Richard (1985).

Jenkins & Daigger

CAUSES

- Waste composition
  - high carbohydrate
  - volatile acids
  - readily degradable substrates

Causes

- Substrate Concentration
  - low substrate concentrations favor filaments due to their low K_S values
- Sludge Loading (Food to Microorganism Ratio)

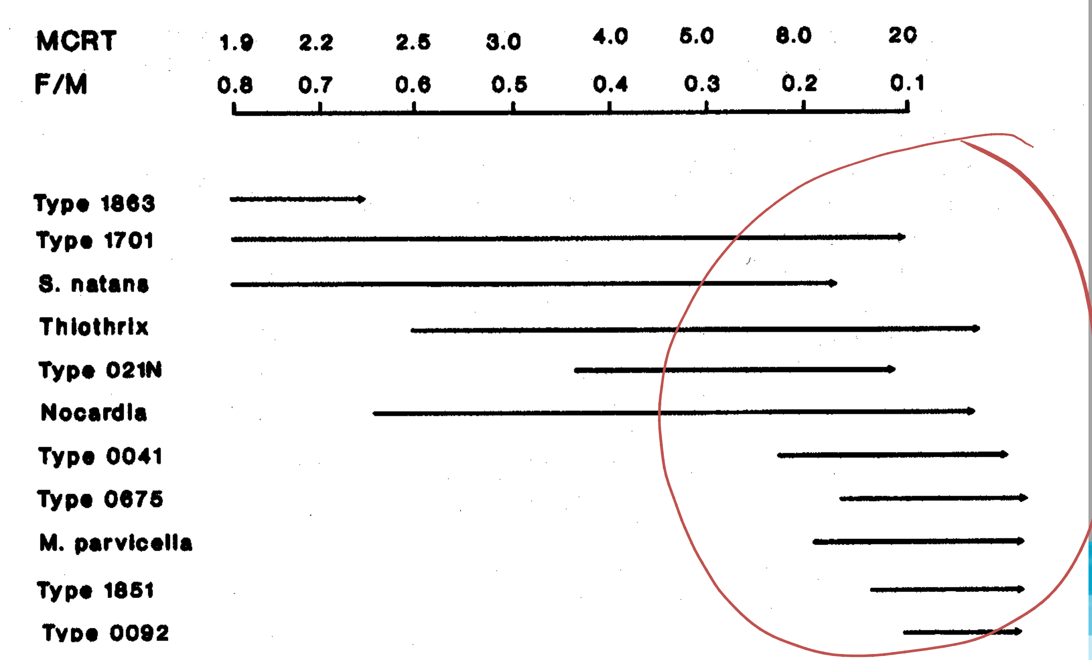
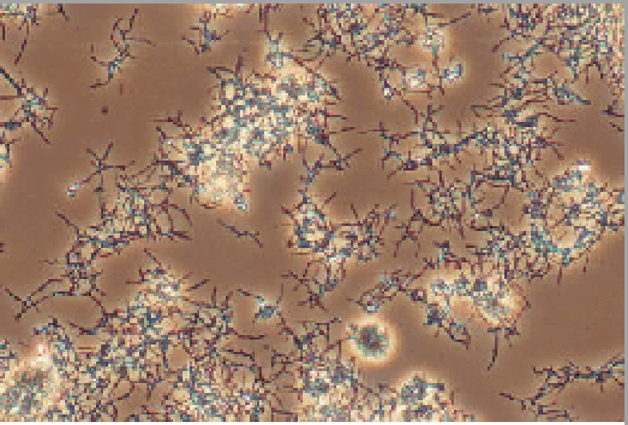


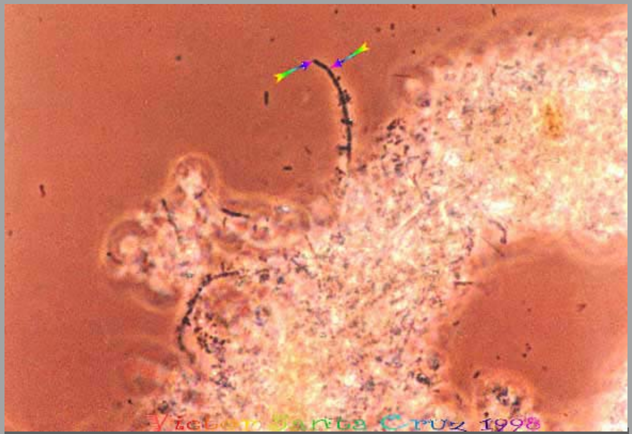
Figure 9.10 Relation of sludge age and F/M ratio to the occurrence of specific filamentous microorganisms in activated sludge. From Jenkins (1992). (Courtesy of Pergamon Press.)

Photos of bulking organisms

Thiothrix spp.

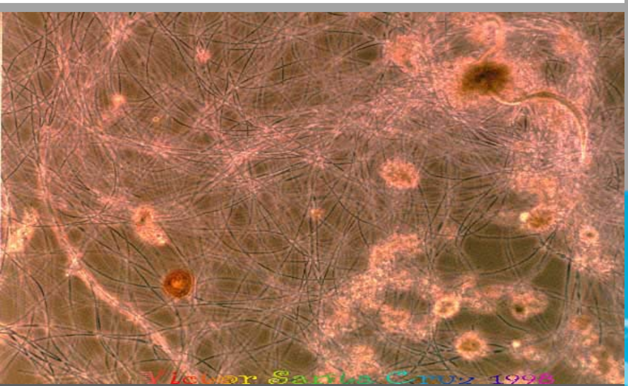


Nocardia Foam (200X)

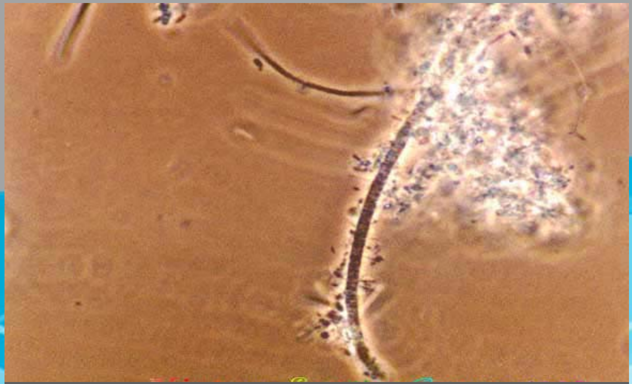


Type 021N

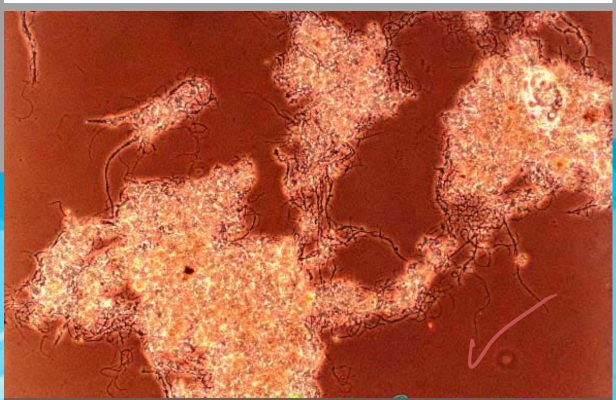
S. natans (1000X)



Type 1701



Type 0041N



Microthrix parvicella

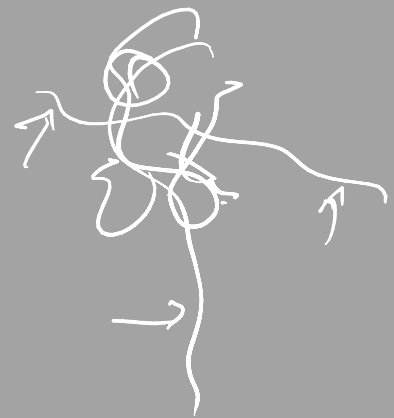
Causes

- pH
  - low________ pH tends to favor filaments
- Sulfide
  - sulfide tends to encourage growth of sulfur________ filaments______________ such as
    - *Thiothrix*
    - *Beggiatoa*
    - 021N
- D.O.
  - *Sphaerotilus natans* K_S for oxygen is 0.01
  - K_S for oxygen for floc formers is 0.15

DO ≥ 2.0

BULKING CONTROL

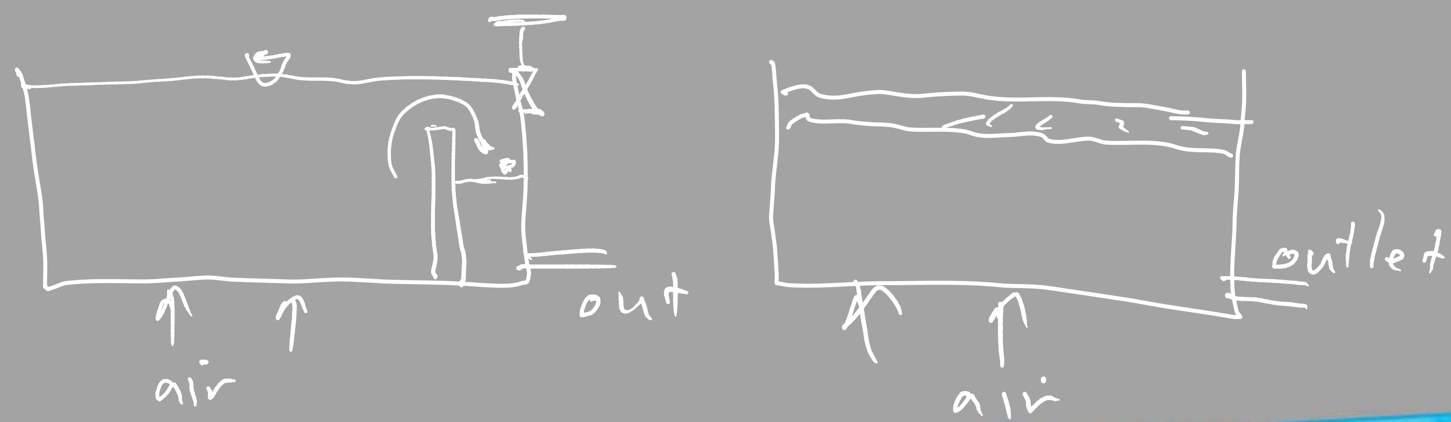
- Chlorinate________ or Ozonate________ of RAS



- Biological Selector
  - aerobic________ selector
  - anoxic________ selector
  - anaerobic________ selector

FOAMING IN ACTIVATED SLUDGE

- Types of activated sludge foams:
  - surface active compounds (surfactants)
  - detergents
  - scum (e.g., as a result of denitrification)
  - actinomycetes foam



Foam Nuisance

- a esthetic ______________ and safety hazard (e.g., slippery walkways)
- increased levels of o rganic ______________ c ompounds ______________ in effluent
- foaming in a naerobic ______________ digesters
- nuisance o rganisms ______________
- opportunistic p athogens ______________ (e.g., *Nocardia asteroides*)

Foam Microbiology

- Actinomycetes _______________ is the most predominant foam causing organism
- Nocardia _______________ is a predominant member of this classification (e.g., *Gordona amarae*, formerly *Nocardia amarae, N. asteroides, N. pinensis*, and *Rhodococcus*).
- *Gordona amarae* and *N. pinensis* are usual suspects
- foaming is problematic when *Nocardia* concentration exceeds 26 _______ mg *Nocardia* per g VSS

Mechanisms of Foam Production

- Gas bubbles______ from aeration or denitrification assist in flotation
- hydrophobic______ nature of cell wall assist in their transport to air-water interface
- biosurfactants______ produced by microorganisms accelerate foaming
- foaming is exacerbated by:
  - long______ SRTs (i.e., >9 d)
  - warm______ temperatures (i.e., >18°C)
  - wastewaters with high fats______ , oil______, and grease______ (FOG)

FOAM CONTROL

- chlorination of foams
- lower SRT
- selector
- reduced airflow
- reduce FOG, pH
- recirculation of anaerobic digester supernatant
- water spray
- antifoam agents
- physical modifications (e.g., overflow weirs)

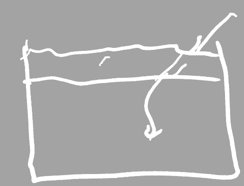


Figure 1. Foaming in two Australian activated sludge plants. Plant on right is an oxidation ditch with foam covering the dividing wall.

http://www.tcd.ie/Centre_for_the_Environment/watertechnology/images/17.2.jpg