Microbiology Methods for Wastewater Water Laboratories

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Thanks to Jeanine Miller-Nelson (Fairfax Water)

Critical Elements for Microbiology

- Personnel
- Laboratory Equipment and Supplies
- General Laboratory Practices
- Analytical Methodology (Reference method)
- Sample Collection, Handling and Preservation
- Quality Assurance
- Records and Data Reporting
- Action Response to Laboratory Results

Personnel

- Analyst
  - Ensure all personnel meets your lab's requirement for analytical testing.
  - Recommend at least two weeks of training for each micro test method, and signed off by trainer.
  - Training on all Quality Control (QC) associated with required micro testing.
  - Passing of Initial Demonstrate of Capability, results on all ten samples passing before analyzing any compliance samples (before reporting data to any regulatory agency).

Laboratory Equipment and Supplies

- pH meter (if needed to prepare media)
  - Accuracy with ± 0.1 standard units
  - Standardize before each use with pH 4.0, 7.0 and 10.0 buffers, recorded each pH and temperature (or other buffer concentrations)
  - Check with 8.0 buffer (or other buffer concentrations)
  - Record slope and all data points and temperatures
  - Maintained according to manufacturer's instructions
- Balance (if needed to prepare media)
  - Readability of 0.1 g
  - Sensitivity of 0.1 g for a load of 150 g and 1 mg for load of 10 g or less (record)
  - Maintenance and calibration performed annually
- Thermometers
  - Graduated in 0.1°C increments for Incubators and Water baths.
  - Graduated in 0.5°C for Refrigerators
  - Calibration or accuracy checked annually (may require correction factor)

Laboratory Equipment and Supplies

- Incubator
  - Must maintain a constant temperature of 35 ± 0.5 °C
  - Record temperature twice each day in use, separated by at least 4 hours

Laboratory Equipment and Supplies

- Autoclave
  - Maintain sterilization temperature during cycle
  - A complete cycle is within 45 minutes, and temp ≥121 for 15 minute and psi ≥ 15 psi for sterilization used for media. The time and temp may depend on the reference method.
  - Record date, contents, sterilization time & temperature, total time in autoclave each use (for media if required)
  - Maintenance performed annually
  - Check automatic timer each quarter with stop watch

Laboratory Equipment and Supplies

- Refrigerator
  - Maintain temperature for samples of >0.0 to <10.0°C (40 CFR Part 136.3 Table II)
  - Temperature for certain media is >2.0 to <8.0°C
  - Record temperature once each day in use
- Inoculating equipment
  - Sterile or presterilized disposable loops, sticks and swabs should be used

Laboratory Equipment and Supplies

- Pipets
  - Pre-sterilized plastic or glass, 10 mL or less must be accurate to within 2.5% (if purchased sterilized pipets check accuracy for each new lot before use with 2.5%)

Glassware and Plasticware

  - Borosilicate glass or clear, non-toxic plastic
  - Tube closures should be screw caps with non-toxic liners, stainless steel, plastic or aluminum
- Sample Containers
  - Wide mouth plastic or glass bottles or sterile plastic bags with sodium thiosulfate. Autoclave sample bottles before using, add sodium thiosulfate if needed (for Colilert method do not need), and autoclave tape on bottle and cap. Autoclave so temp ≥121 for 15 minute and psi ≥ 15 psi.
  - **Method Specific Supplies**
  - See reference test methods for specific equipment and supplies. For example testing for Colilert method supplies and equipment: colilert media, quanti-trays, containers with sodium thiosulfate, UV lamp, sealer, incubator, sterile water, andQuanti Cult Kit to check new colilert media lot before first use.

General Laboratory Practices

- Sterilization procedures
  - Autoclave at 121°C for recommended time at least 15 psi
  - Media should be removed immediately after end of cycle
- Sample Containers
  - Randomly test 1 container from each batch or lot for sterility using 25 mL non-selective broth (such as Tryptic Soy Broth), incubate for 48 hours & check for growth and/or follow specific procedures from test method used.
- Reagent grade water
  - Quality should meet criteria for Conductivity, Metals, Total Chlorine Residual, HPC and Bacteriological Quality
- Glassware/plasticware washing
  - Clean with brush and, soap & water, and distilled or deionized water for final rinsing
  - Check for acid or alkalinity after washing a batch of glassware/plasticware (or at lest daily) using a suitable pH indicator such as bromothymol blue solution.
  - Inhibitory residue test should be performed prior to initial use of detergent lot (and annually), or whenever different washing procedure or detergent is used

Analytical Methodology

- Media
  - Discard media by manufacturer's expiration date
  - Store prepared medium in dark at recommended temperature
  - For lab-prepared media, record date prepared, type, lot #, volume, sterilization time & temperature, final pH and initials
  - For commercially-prepared media, record date received, type, lot # and pH verification if specified by method
  - Each batch of lab-prepared and each lot of commercially-prepared media should be checked before use for sterility and with positive & negative control cultures
- Examples of control cultures:
  - Total coliforms
    - Positive: *Escherichia coli* Negative: *Pseudomonas aeruginosa*
  - Fecal coliforms
    - Positive: *Escherichia coli* Negative: *Enterobacter aerogenes*
  - *E. coli*
    - Positive: *Escherichia coli* Negative: *Pseudomonas aeruginosa*

Analytical Methodology

- Analysis
  - For compliance samples, use only methods approved in 40 CFR Part 136.3
  - Shake samples at least 25 times before analyzing, or time specified in reference method
  - Tolerance of dilution buffer should be ± 2 mL for volumes of 90 or 99 mL

Sample Collection, Handling and Preservation

- Sample collectors should be trained in aseptic sampling procedures
- Sampling
  - Locations must be Outfall locations or representative of Outfall location
- Sample Holding/Travel Time (all times are from collection to placing sample in incubator)
  - Total coliforms, Fecal Coliforms, and E.coli for wastewater: 8 hours
- Sample Icing
  - Recommended to hold samples at < 10 °C during transport

Quality Assurance

- Laboratory should prepare & follow a Quality Assurance (QA) Manual and SOPs
- Analyze a set of Proficiency Testing (PT) samples twice every 12 months (once every 6months ± 1 month) for <u>each method</u> for which laboratory is certified

Records and Data Reporting

- Laboratory should keep thorough and accurate records
- QA Manual should describe procedures used for record retention
- Laboratory should maintain easily accessible records for 5 years (includes raw data, calculations and QC data)
- Electronic data should be backed up

Records and Data Reporting

- Data should be recorded in ink; changes should be lined through and dated & initialed
- Record date & time of sample receipt and name of person receiving sample, along with any comments on sample condition
- Record laboratory sample identification, date & time analysis begins, initials of analyst, method used, items noted as QC and results
- Maintain preventive maintenance and repair records for all instruments and equipment for five years

Basic Aseptic Technique

- Disinfect bench tops prior to analysis
- Long hair should be pulled back
- Wash hands prior to analysis
- Take every precaution not to contaminate sterile plates or media during analysis by talking, coughing, sneezing, etc
- Do not touch any surfaces that are sterile (interiors or plates, tips of pipets, etc)
- Keep sterile plates, bottles, etc closed until ready to use
- Use care when removing presterilized pipets from bulk bags, to avoid contaminating inside of bag

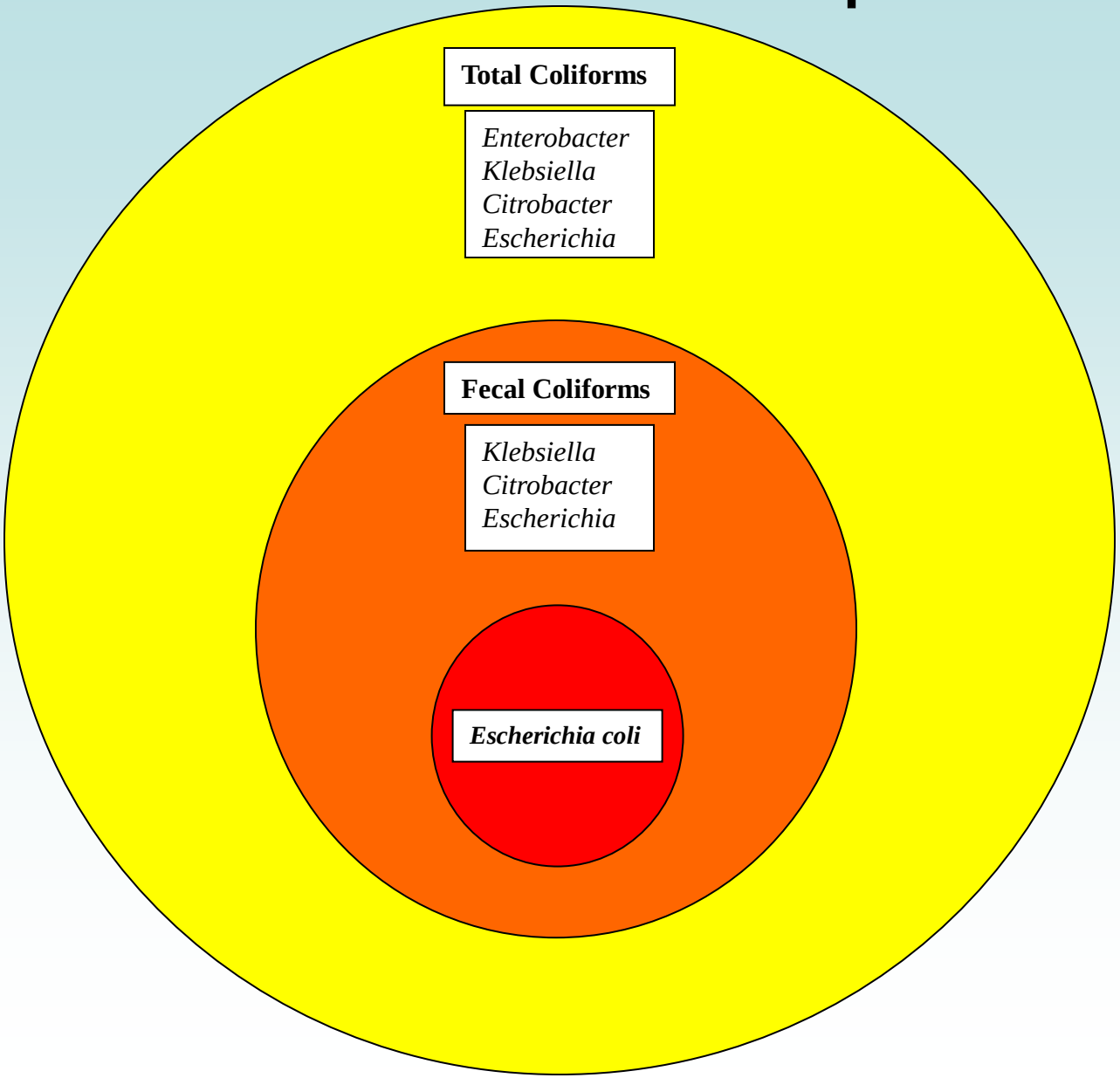
Questions?

Total Coliforms/E. coli by Colilert® and Quanti-Tray®

Coliforms

The term total coliforms refers to a broad group of bacteria that belong to the taxonomic family *Enterobacteriaceae*. This group includes the genera *Enterobacter*, *Klebsiella*, *Citrobacter*, and *Escherichia*. Total coliforms are present in the environment and are used as an indicator to determine the efficacy of treatment plant operation.

Coliform Group



Escherichia coli

Escherichia coli (commonly abbreviated as *E. coli*) is a member of the fecal coliform group. *E. coli* is found in the digestive tract of warm blooded animals and is considered an indicator of fecal contamination..

Total Coliforms/E. coli by Colilert® and Quanti-Tray®

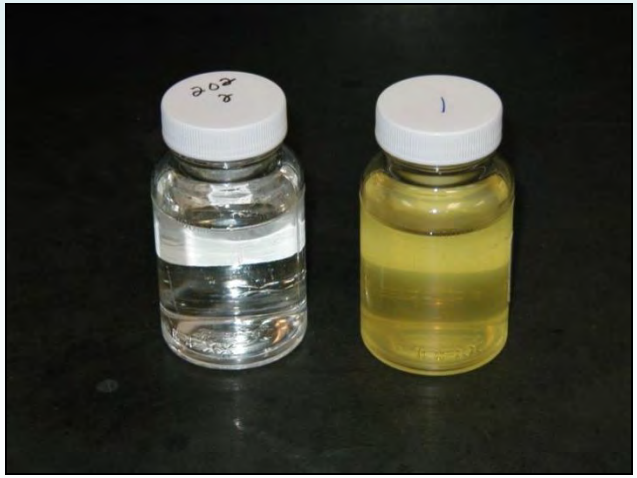
Method Summary

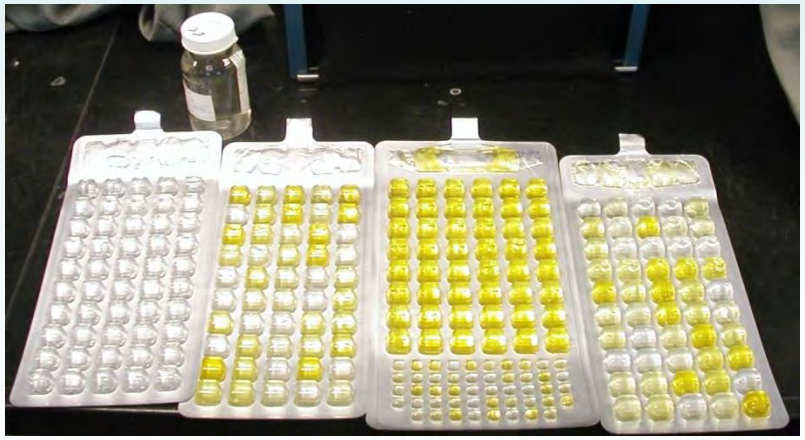
These methods allow for the simultaneous detection of total coliform bacteria and *E. coli* through the use of nutrient indicators ortho-nitophenyl-β-D-galactopyranoside (ONPG) and 4-methyl-umbelliferyl-β-D-glucuronide (MUG). Coliform bacteria and *E. coli* produce enzymes that can metabolize ONPG and MUG respectively.

Total Coliforms/E. coli by Colilert® and Quanti-Tray®

Method Summary

- When ONPG is metabolized, a yellow color change occurs, indicating the presence of total coliforms.

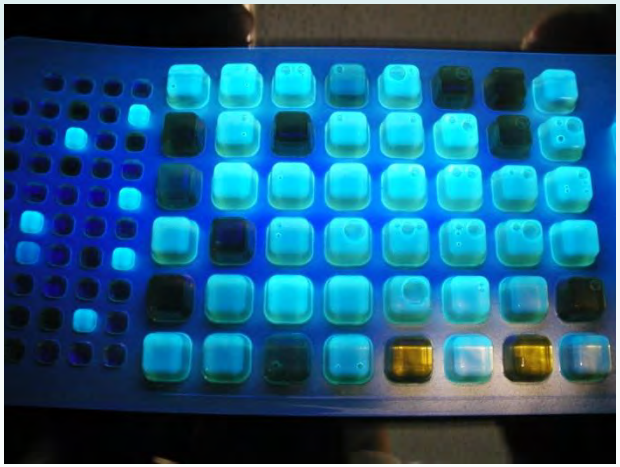




Total Coliforms/E. coli by Colilert® and Quanti-Tray®

Method Summary

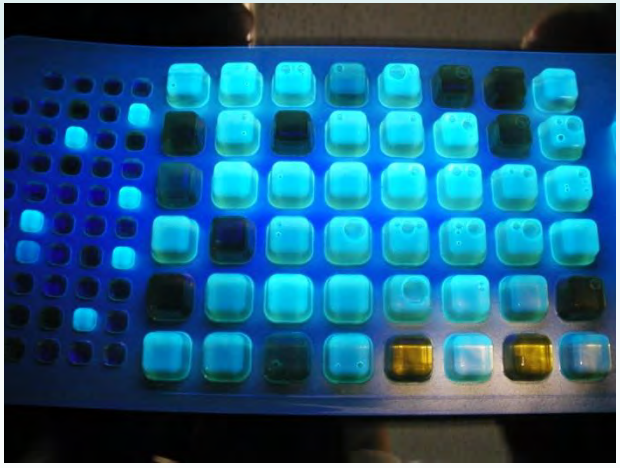
- When MUG is metabolized, a fluorescent product is produced, indicating the presence of *E. coli*.



Total Coliforms/E. coli by Colilert® and Quanti-Tray®

Method Summary

- Colilert® is a Presence/Absence test.
- Quanti-Tray® is an enumeration test.



Total Coliforms/E. coli by Colilert® and Quanti-Tray®

Helpful Hints

- Make sure media is not discolored or caking before opening snap pack.
- If a sample is yellow or fluorescing, but is less intense than the comparator, it can be reincubated, but do not exceed a total of 28 hours incubation.

Total Coliforms/E. coli by Colilert® and Quanti-Tray®
Interferences

- Some non-coliform bacteria produce small amounts of the enzyme β-D-galactosidase. These bacteria are suppressed, and will not produce a positive response unless 10^4 CFU/mL are present.
- A combination of hydrogen sulfide and high levels of either manganese or iron may cause a greenish-black to black color change to occur after incubation.
- Some water samples may have background color. If there is background color, compare the inoculated sample to a control containing only sample.

Total Coliforms/E. coli by Colilert® and Quanti-Tray®
<u>Interferences</u>

- Some samples may fluoresce without having the yellow color. According to the manufacturer, an ONPG(-)/ MUG(+) test (colorless with fluorescence) is considered a nonspecific reaction as it does not meet the test definition of a total coliform or *E. coli*. Therefore this test would be interpreted as negative for both total coliforms and *E. coli*. Per the method, only samples that test positive for total coliforms should be checked under UV light for *E. coli*.

Total Coliforms/E. coli by Colilert® and Quanti-Tray®

- Colilert® media QC requirements
  - Media Fluorescence Check
    - Each lot of media must be checked for fluorescence prior to use.
    - Media dissolved in sterile reagent grade water should not fluoresce.
  - Positive/Negative Control Check
    - Each lot of media must be checked for selectivity and proper performance prior to use, and every 3 months while in use. Media dissolved in sterile reagent grade water and inoculated with the following cultures should exhibit the appropriate reactions after a 24 hour incubation at 35 ± 0.5 °C.

Culture	Reaction
E. coli	Positive yellow color, fluorescence
K. pneumoniae or *E. aerogenes*	Positive yellow color, no fluorescence
P. aeruginosa	No color, no fluorescence

Total Coliforms/E. coli by Colilert® and Quanti-Tray®

- Vessel QC requirements
  - Vessel sterility check
    - Each lot of vessels must be checked for sterility prior to use.
    - Add 25mL of single strength Tryptic Soy Broth to a randomly selected vessel and incubate for 48 hours at 35 ± 0.5 °C.
    - If no growth is present, the vessel lot may be put into service.
  - Vessel volume check
    - Each lot of vessels must be checked for volume accuracy prior to use.
    - Fill a Class A 100mL graduated cylinder with reagent grade water and transfer to a randomly selected vessel.
    - The level of water should be at the 100mL mark on the vessel for the lot to be placed in service.
  - Vessel fluorescence check
    - A randomly selected vessel should be checked for fluorescence using the UV light box prior to use.
    - Vessel should not exhibit any fluorescence.

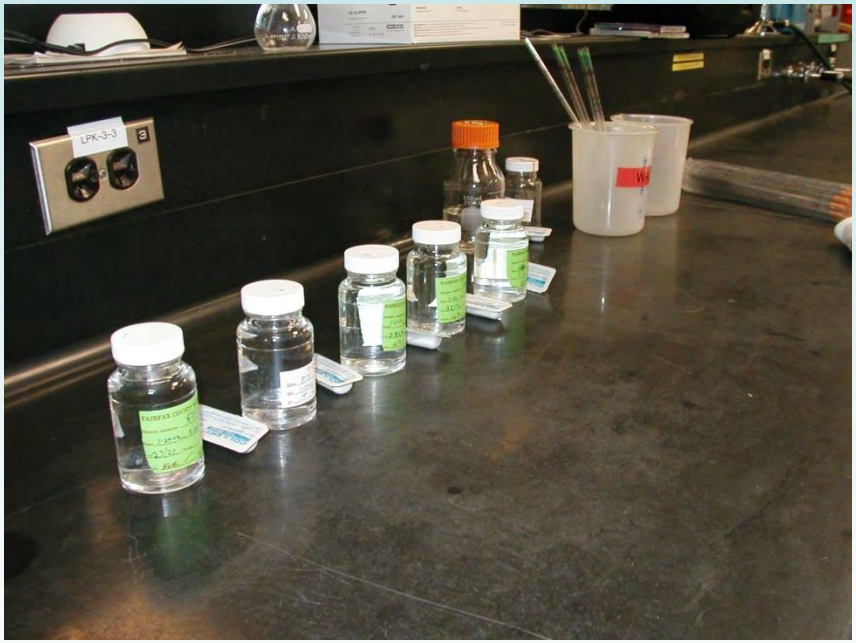
Total Coliforms/E. coli by Colilert® and Quanti-Tray®

- Quanti-Tray® QC requirements
  - Quanti-Tray® sterility check
    - Each lot must be checked for sterility prior to use.
    - Add 25mL of single strength tryptic soy broth to a randomly selected Quanti-Tray®, seal, and incubate for 48 hours at 35 ± 0.5 °C.
    - If no growth is present, the Quanti-Tray® lot may be put into service.
  - Quanti-Tray® Sealer QC check
    - The sealer is checked each month for proper sealing.
    - Add 100mL of a solution of water and a dark dye to a Quanti-Tray®, and run through the sealer. Check for leaks between the wells and on edges of the tray.

Total Coliforms/E. coli by Colilert®

Total Coliforms/E. coli by Colilert®

- Materials:
  - Sterile sample vessels
  - Colilert® media
  - Sterile Reagent Grade Water
  - Sterile pipettes
  - Vessel rack
  - Incubator, 35 ± 0.5 ºC
  - UV light

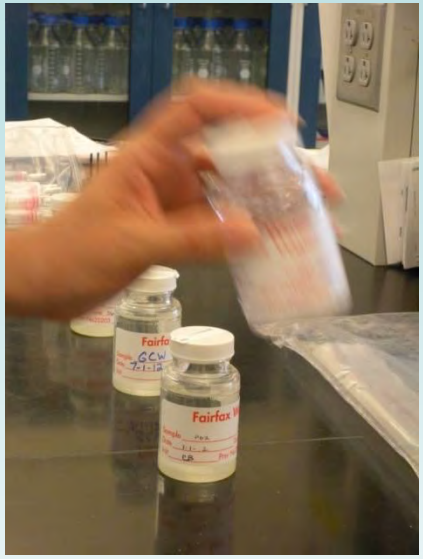


Total Coliforms/E. coli by Colilert®

- Shake sample thoroughly (at least 25 times)
- Use sterile pipet to draw volume of sample down to 100 mL.
- Tap Colilert® media snap pack on counter to settle contents. Snap pack open, and empty media into sample vessel.

Total Coliforms/E. coli by Colilert®

- Replace cap and shake sample vigorously. Media does not have to be completely dissolved in the sample prior to incubation.
- Place samples in a rack, and incubate at 35 ± 0.5 °C for 24-28 hours.



Fairfax Water

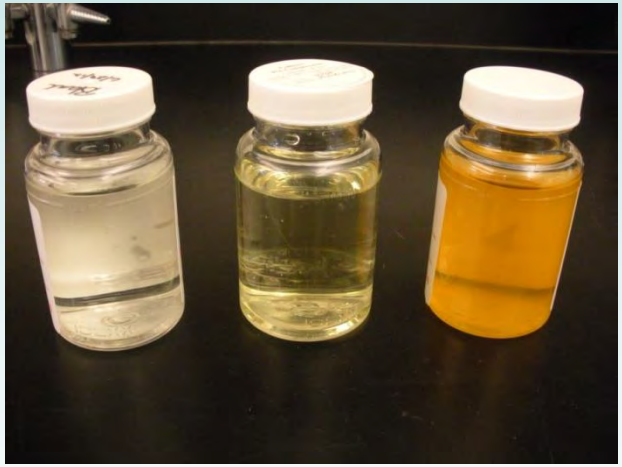
Total Coliforms/E. coli by Colilert®

- After incubating for 24 – 28 hours, the bottles are read using a Colilert® Presence/Absence comparator for reference.

Fairfax Water

Total Coliforms/E. coli by Colilert®

- Samples that show a yellow color equal to or greater than that of the Comparator are total coliform positive.
- Samples with no color are total coliform negative.



Total Coliforms/E. coli by Colilert®

- Total coliform positive (yellow) samples and the Comparator are checked with UV light.
- Samples that show fluorescence equal to or greater than that of the Comparator are *E. coli* positive.
- Samples with no fluorescence are *E. coli* negative.

Total Coliforms/E. coli by Colilert®

Questions?

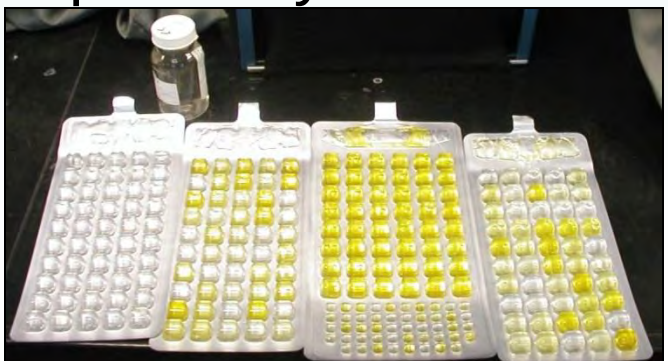
Total Coliform/E. coli by Quanti-Tray®

Total Coliform/E. coli by Quanti-Tray®

This method is used for the detection of total coliform bacteria and *E. coli* in source water and ground water. This method is approved for the analysis of samples for Long Term 2 Enhanced Surface Water Treatment Rule (LT2) and Ground Water Rule compliance.

Total Coliform/E. coli by Quanti-Tray®

- Quanti-Tray® trays are available in two different sizes
  - The 51-well Quanti-Tray® contains only large wells, and is appropriate for samples containing less than 200.5 MPN (Most Probable Number) per 100 mL.
  - The 97-well Quanti-Tray®/2000 contains 49 large and 48 small wells. This tray is appropriate for samples containing less than 2419.6 MPN (Most Probable Number) per 100 mL.
- Samples may be diluted as needed



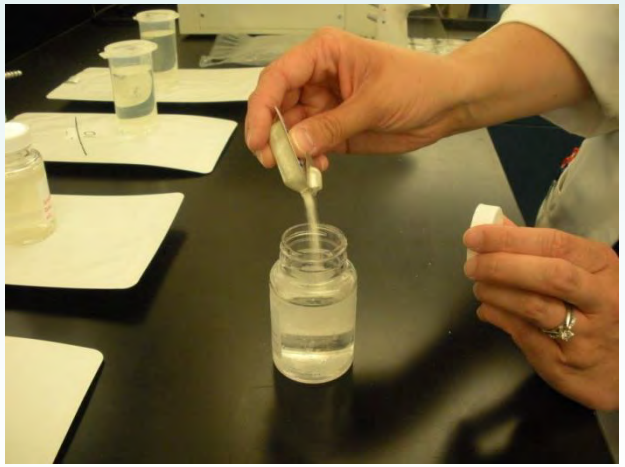
Total Coliform/E. coli by Quanti-Tray®

- Materials:
  - Package of sterile Quanti-Tray® trays
  - Sterile sample bottles
  - Colilert® media
  - Sterile dilution water (90 and 99 mL)
  - Sterile pipettes
  - Quanti-Tray® sealer and inserts
  - Incubator, 35 ± 0.5 ºC
  - UV light
  - MPN table

Total Coliform/E. coli by Quanti-Tray®

Procedure

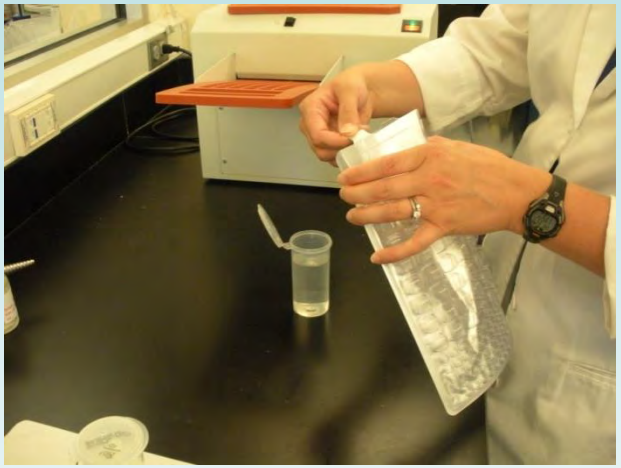
- Preheat Quanti-Tray® sealer.
- Shake sample thoroughly (at least 25 times)
- Use sterile pipet to draw volume of sample down to 100 mL. Sample may be diluted using sterile deionized water if necessary.
- Tap Colilert® media snap pack on counter to settle contents. Snap pack open, and empty media into sample bottle.

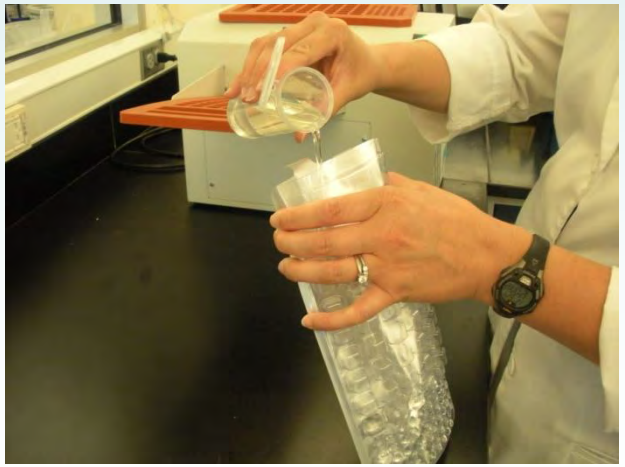


Total Coliform/E. coli by Quanti-Tray®

Procedure

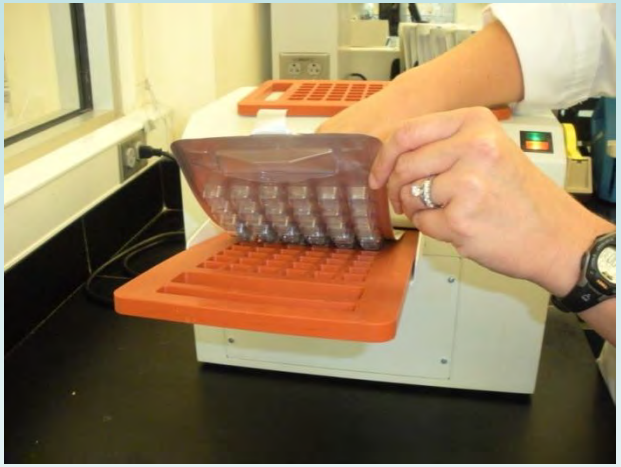
- Replace cap and mix sample until media dissolves. Media needs to be completely dissolved in the sample prior to pouring into the Quanti-Tray®.
- Open Quanti-Tray® by squeezing in at the sides while pulling the foil tab away. Pour sample into tray.

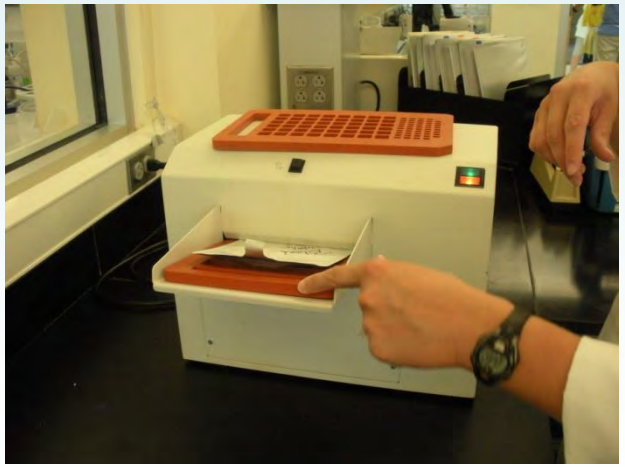




Total Coliform/E. coli by Quanti-Tray®
Procedure

- Fit Quanti-Tray® in rubber insert and slide into the preheated sealer.





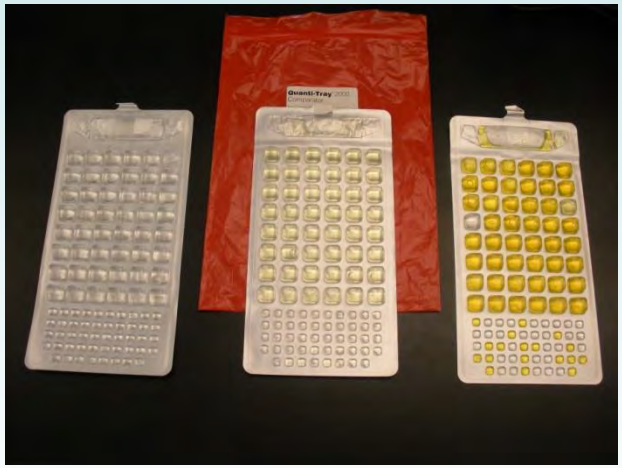
Total Coliform/E. coli by Quanti-Tray®
Procedure

- The Quanti-Tray® will go through the sealer and the sealed Quanti-Tray® will come out the back.
- Incubate sealed trays at 35 ± 0.5 °C for 24-28 hours.

Total Coliform/E. coli by Quanti-Tray®

Reading Samples

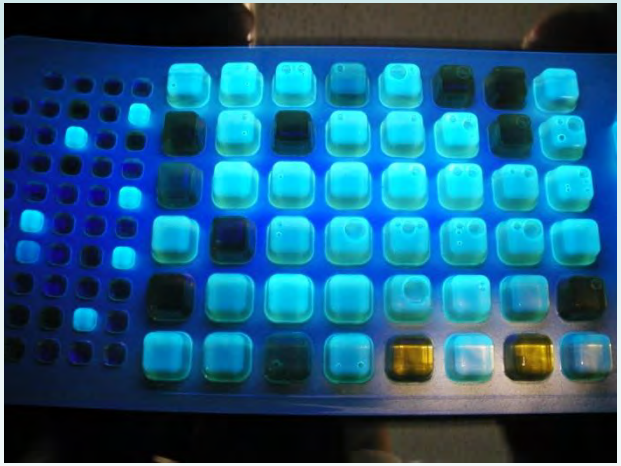
- Wells with a yellow color equal to or greater than that of the Quanti-Tray® Comparator are total coliform positive.
- Count the number of positive wells, and refer to the provided Quanti-Tray® MPN table to find the total coliform MPN.



Total Coliform/E. coli by Quanti-Tray®

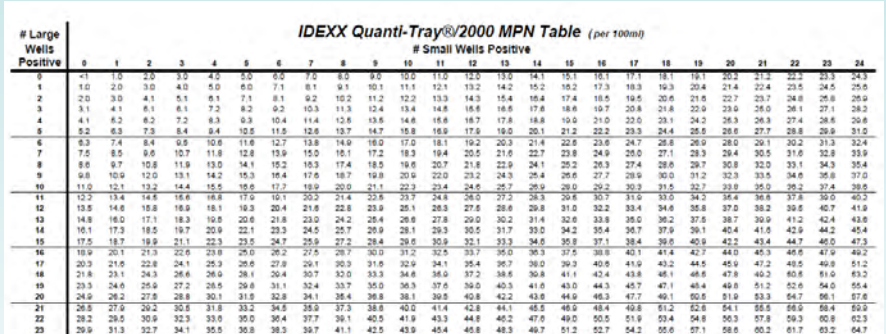
Reading Samples

- Trays with total coliform positive (yellow) wells and the Comparator are checked for fluorescence with a UV light.
- Wells that show fluorescence equal to or greater than that of the Comparator are *E. coli* positive.
- Count the number of positive wells, and refer to the provided MPN table to find the *E. coli* MPN.
- The number of positive wells is converted to a Most Probable Number result in the Quanti-Tray® or Quanti-Tray®/2000 MPN Table. The MPN is multiplied by the appropriate dilution factor, if needed.



Total Coliform/E. coli by Quanti-Tray®

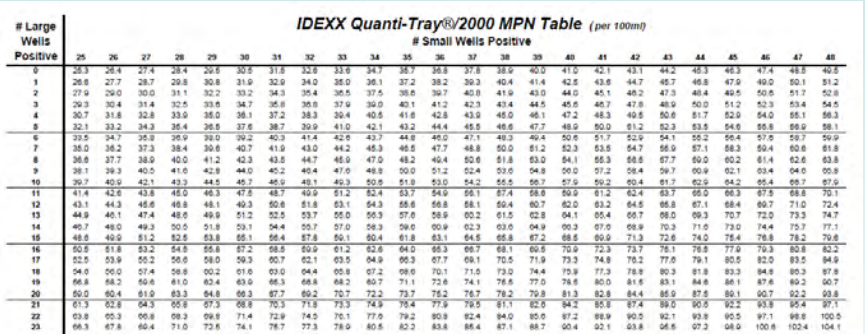
MPN table for Quanti-Tray®/2000



IDEXX Quanti-Tray®/2000 MPN Table *(per 100ml)*

# Large Wells Positive	# Small Wells Positive																								
	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24
0	<1	1.0	2.0	3.0	4.0	5.0	6.0	7.0	8.0	9.0	10.0	11.0	12.0	13.0	14.1	15.1	16.1	17.1	18.1	19.1	20.2	21.2	22.2	23.3	24.3
1	1.0	2.0	3.0	4.0	5.0	6.0	7.1	8.1	9.1	10.1	11.1	12.1	13.2	14.2	15.2	16.2	17.3	18.3	19.3	20.4	21.4	22.4	23.5	24.5	25.6
2	2.0	3.0	4.1	5.1	6.1	7.1	8.1	9.2	10.2	11.2	12.2	13.3	14.3	15.4	16.4	17.4	18.5	19.5	20.6	21.6	22.7	23.7	24.8	25.8	26.9
3	3.1	4.1	5.1	6.1	7.2	8.2	9.2	10.3	11.3	12.4	13.4	14.5	15.6	16.6	17.6	18.6	19.7	20.8	21.8	22.9	23.9	25.0	26.1	27.1	28.2
4	4.1	5.2	6.2	7.2	8.3	9.3	10.4	11.4	12.5	13.5	14.6	15.6	16.7	17.8	18.8	19.9	21.0	22.0	23.1	24.2	25.3	26.3	27.4	28.5	29.6
5	5.2	6.3	7.3	8.4	9.4	10.5	11.5	12.6	13.7	14.7	15.8	16.9	17.9	19.0	20.1	21.2	22.2	23.3	24.4	25.5	26.6	27.7	28.8	29.9	31.0
6	6.3	7.4	8.4	9.5	10.6	11.6	12.7	13.8	14.9	16.0	17.0	18.1	19.2	20.3	21.4	22.5	23.6	24.7	25.8	26.9	28.0	29.1	30.2	31.3	32.4
7	7.5	8.5	9.6	10.7	11.8	12.8	13.9	15.0	16.1	17.2	18.3	19.4	20.5	21.6	22.7	23.8	24.9	26.0	27.1	28.3	29.4	30.5	31.6	32.8	33.9
8	8.6	9.7	10.8	11.9	13.0	14.1	15.2	16.3	17.4	18.5	19.6	20.7	21.8	22.9	24.1	25.2	26.3	27.4	28.6	29.7	30.8	32.0	33.1	34.3	35.4
9	9.8	10.9	12.0	13.1	14.2	15.3	16.4	17.6	18.7	19.8	20.9	22.0	23.2	24.3	25.4	26.6	27.7	28.9	30.0	31.2	32.3	33.5	34.6	35.8	37.0
10	11.0	12.1	13.2	14.4	15.5	16.6	17.7	18.9	20.0	21.1	22.3	23.4	24.6	25.7	26.9	28.0	29.2	30.3	31.5	32.7	33.8	35.0	36.2	37.4	38.5
11	12.2	13.4	14.5	15.6	16.8	17.9	19.1	20.2	21.4	22.6	23.7	24.8	26.0	27.2	28.3	29.5	30.7	31.9	33.0	34.2	35.4	36.6	37.8	39.0	40.2
12	13.5	14.6	15.8	16.9	18.1	19.3	20.4	21.6	22.8	23.9	25.1	26.3	27.5	28.6	29.8	31.0	32.2	33.4	34.6	35.8	37.0	38.2	39.5	40.7	41.9
13	14.8	16.0	17.1	18.3	19.5	20.6	21.8	23.0	24.2	25.4	26.6	27.8	29.0	30.2	31.4	32.6	33.8	35.0	36.2	37.5	38.7	39.9	41.2	42.4	43.6
14	16.1	17.3	18.5	19.7	20.9	22.1	23.3	24.5	25.7	26.9	28.1	29.3	30.5	31.7	33.0	34.2	35.4	36.7	37.9	39.1	40.4	41.6	42.9	44.2	45.4
15	17.5	18.7	19.9	21.1	22.3	23.5	24.7	25.9	27.2	28.4	29.6	30.9	32.1	33.3	34.6	35.8	37.1	38.4	39.6	40.9	42.2	43.4	44.7	46.0	47.3
16	18.9	20.1	21.3	22.6	23.8	25.0	26.2	27.5	28.7	30.0	31.2	32.5	33.7	35.0	36.3	37.5	38.8	40.1	41.4	42.7	44.0	45.3	46.6	47.9	49.2
17	20.3	21.6	22.8	24.1	25.3	26.6	27.8	29.1	30.3	31.6	32.9	34.1	35.4	36.7	38.0	39.3	40.6	41.9	43.2	44.5	45.9	47.2	48.5	49.8	51.2
18	21.8	23.1	24.3	25.6	26.9	28.1	29.4	30.7	32.0	33.3	34.6	35.9	37.2	38.5	39.8	41.1	42.4	43.8	45.1	46.5	47.8	49.2	50.6	51.9	53.3
19	23.3	24.6	25.9	27.2	28.5	29.8	31.1	32.4	33.7	35.0	36.3	37.6	39.0	40.3	41.6	43.0	44.3	45.7	47.1	48.4	49.8	51.2	52.6	54.0	55.4
20	24.9	26.2	27.5	28.8	30.1	31.5	32.8	34.1	35.4	36.8	38.1	39.5	40.8	42.2	43.6	44.9	46.3	47.7	49.1	50.5	51.9	53.3	54.7	56.1	57.6
21	26.5	27.9	29.2	30.6	31.9	33.2	34.5	35.9	37.3	38.6	40.0	41.4	42.8	44.1	45.5	46.9	48.4	49.8	51.2	52.6	54.1	55.5	56.9	58.4	59.9
22	28.2	29.5	30.9	32.3	33.6	35.0	36.4	37.7	39.1	40.5	41.9	43.3	44.8	46.2	47.6	49.0	50.5	51.9	53.4	54.8	56.3	57.8	59.3	60.8	62.3
23	29.9	31.3	32.7	34.1	35.5	36.8	38.3	39.7	41.1	42.5	43.9	45.4	46.8	48.3	49.7	51.2	52.7	54.2	55.6	57.1	58.6	60.2	61.7	63.2	64.7
24	31.7	33.1	34.5	35.9	37.3	38.8	40.2	41.7	43.1	44.6	46.0	47.5	49.0	50.5	52.0	53.5	55.0	56.5	58.0	59.5	61.1	62.6	64.2	65.8	67.3
25	33.6	35.0	36.4	37.9	39.3	40.8	42.2	43.7	45.2	46.7	48.2	49.7	51.2	52.7	54.3	55.8	57.3	58.9	60.5	62.0	63.6	65.2	66.8	68.4	70.0
26	35.5	36.9	38.4	39.9	41.4	42.8	44.3	45.9	47.4	48.9	50.4	52.0	53.5	55.1	56.7	58.2	59.8	61.4	63.0	64.7	66.3	67.9	69.6	71.2	72.9
27	37.4	38.9	40.4	42.0	43.5	45.0	46.5	48.1	49.6	51.2	52.8	54.4	56.0	57.6	59.2	60.8	62.4	64.1	65.7	67.4	69.1	70.8	72.5	74.2	75.9
28	39.5	41.0	42.6	44.1	45.7	47.3	48.8	50.4	52.0	53.6	55.2	56.9	58.5	60.2	61.8	63.5	65.2	66.9	68.6	70.3	72.0	73.7	75.5	77.3	79.0
29	41.7	43.2	44.8	46.4	48.0	49.6	51.2	52.8	54.5	56.1	57.8	59.5	61.2	62.9	64.6	66.3	68.0	69.8	71.5	73.3	75.1	76.9	78.7	80.6	82.4
30	43.9	45.5	47.1	48.7	50.4	52.0	53.7	55.4	57.1	58.8	60.5	62.2	64.0	65.7	67.5	69.3	71.0	72.9	74.7	76.5	78.3	80.2	82.1	84.0	85.9
31	46.2	47.9	49.5	51.2	52.9	54.6	56.3	58.1	59.8	61.6	63.3	65.1	66.9	68.7	70.5	72.4	74.2	76.1	78.0	79.9	81.8	83.7	85.7	87.6	89.6
32	48.7	50.4	52.1	53.8	55.6	57.3	59.1	60.9	62.7	64.5	66.3	68.2	70.0	71.9	73.8	75.7	77.6	79.5	81.5	83.5	85.4	87.5	89.5	91.5	93.6
33	51.2	53.0	54.8	56.5	58.3	60.2	62.0	63.8	65.7	67.6	69.5	71.4	73.3	75.2	77.2	79.2	81.2	83.2	85.2	87.3	89.3	91.4	93.6	95.7	97.9
34	53.9	55.7	57.6	59.4	61.3	63.1	65.0	67.0	68.9	70.8	72.8	74.8	76.8	78.8	80.8	82.9	85.0	87.1	89.2	91.4	93.5	95.7	97.9	100.2	102.4
35	56.8	58.6	60.5	62.4	64.4	66.3	68.3	70.3	72.3	74.3	76.3	78.4	80.5	82.6	84.7	86.9	89.1	91.3	93.5	95.7	98.0	100.3	102.6	105.0	107.3
36	59.8	61.7	63.7	65.7	67.7	69.7	71.7	73.8	75.9	78.0	80.1	82.3	84.6	86.7	88.9	91.2	93.5	95.8	98.1	100.5	102.9	105.3	107.7	110.2	112.7
37	62.9	65.0	67.0	69.1	71.2	73.3	75.4	77.6	79.8	82.0	84.2	86.5	88.8	91.1	93.4	95.8	98.2	100.6	103.1	105.6	108.1	110.7	113.3	115.9	118.6
38	66.3	68.4	70.6	72.7	74.9	77.1	79.4	81.6	83.9	86.2	88.6	91.0	93.4	95.8	98.3	100.8	103.4	105.9	108.6	111.2	113.9	116.6	119.4	122.2	125.0
39	70.0	72.2	74.4	76.7	78.9	81.3	83.6	86.0	88.4	90.9	93.4	95.9	98.4	101.0	103.6	106.3	109.0	111.9	114.7	117.4	120.3	123.2	126.1	129.2	132.2
40	73.9	76.2	78.5	80.9	83.3	85.7	88.2	90.8	93.3	95.9	98.5	101.2	103.9	106.7	109.5	112.4	115.3	118.2	121.2	124.3	127.4	130.5	133.7	137.0	140.3
41	78.0	80.5	83.0	85.5	88.0	90.6	93.3	95.9	98.7	101.4	104.3	107.1	110.0	113.0	116.0	119.1	122.2	125.4	128.7	132.0	135.4	138.8	142.3	145.9	149.5
42	82.6	85.2	87.8	90.5	93.2	96.0	98.8	101.7	104.6	107.6	110.6	113.7	116.9	120.1	123.4	126.7	130.1	133.6	137.2	140.8	144.5	148.3	152.2	156.1	160.2
43	87.8	90.4	93.2	96.0	99.0	101.9	105.0	108.1	111.2	114.5	117.8	121.1	124.6	128.1	131.7	135.4	139.1	143.0	147.0	151.0	155.2	159.4	163.8	168.2	172.8
44	93.1	96.1	99.1	102.2	105.4	108.6	111.9	115.3	118.7	122.3	126.0	129.6	133.4	137.4	141.4	145.6	149.7	154.1	158.6	163.1	167.7	172.7	177.7	182.9	188.2
45	99.3	102.5	105.8	109.2	112.6	116.2	119.8	123.6	127.4	131.4	135.4	139.6	143.9	148.3	152.9	157.6	162.4	167.4	172.6	178.0	183.5	189.2	195.1	201.2	207.5
46	106.3	109.8	113.4	117.2	121.0	126.0	129.1	133.3	137.6	142.1	146.7	151.6	156.6	161.8	167.0	172.5	178.2	184.2	190.4	196.9	203.6	210.5	217.8	225.4	233.3
47	114.3	118.3	122.4	126.6	130.9	135.4	140.1	145.0	150.0	155.3	160.7	166.4	172.3	178.5	185.0	191.8	198.9	206.4	214.2	222.4	231.0	240.0	249.5	259.5	270.0
48	123.9	128.4	133.1	137.9	143.0	148.3	153.9	159.7	165.8	172.2	178.9	185.9	193.5	201.4	209.8	218.7	228.2	238.2	248.9	260.3	272.3	285.1	298.7	313.0	328.2
49	135.5	140.8	146.4	152.3	158.5	165.0	172.0	179.3	187.2	195.6	204.6	214.3	224.7	235.9	248.1	261.3	275.5	290.9	307.6	325.5	344.8	365.4	387.3	410.6	435.2

09-63236-01



IDEXX Quanti-Tray®/2000 MPN Table *(per 100ml)*

# Large Wells Positive	# Small Wells Positive																								
	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40	41	42	43	44	45	46	47	48	
0	25.3	26.4	27.4	28.4	29.5	30.5	31.5	32.6	33.6	34.7	35.7	36.8	37.8	38.9	40.0	41.0	42.1	43.1	44.2	45.3	46.3	47.4	48.5	49.5	
1	26.8	27.7	28.7	29.8	30.8	31.9	32.9	34.0	35.0	36.1	37.2	38.2	39.3	40.4	41.4	42.5	43.6	44.7	45.7	46.8	47.9	49.0	50.1	51.2	
2	27.9	29.0	30.0	31.1	32.2	33.2	34.3	35.4	36.5	37.5	38.6	39.7	40.8	41.9	43.0	44.0	45.1	46.2	47.3	48.4	49.5	50.6	51.7	52.8	
3	29.3	30.4	31.4	32.5	33.6	34.7	35.8	36.8	37.9	39.0	40.1	41.2	42.3	43.4	44.5	45.6	46.7	47.8	48.9	50.0	51.2	52.3	53.4	54.5	
4	30.7	31.8	32.8	33.9	35.0	36.1	37.2	38.3	39.4	40.5	41.6	42.8	43.9	45.0	46.1	47.2	48.3	49.5	50.6	51.7	52.9	54.0	55.1	56.3	
5	32.1	33.2	34.3	35.4	36.5	37.6	38.7	39.9	41.0	42.1	43.2	44.4	45.5	46.6	47.7	48.9	50.0	51.2	52.3	53.5	54.6	55.8	56.9	58.1	
6	33.5	34.7	35.8	36.9	38.0	39.2	40.3	41.4	42.6	43.7	44.8	46.0	47.1	48.3	49.4	50.6	51.7	52.9	54.1	55.2	56.4	57.6	58.7	59.9	
7	35.0	36.2	37.3	38.4	39.6	40.7	41.9	43.0	44.2	45.3	46.5	47.7	48.8	50.0	51.2	52.3	53.5	54.7	55.9	57.1	58.3	59.4	60.6	61.8	
8	36.6	37.7	38.9	40.0	41.2	42.3	43.5	44.7	45.9	47.0	48.2	49.4	50.6	51.8	53.0	54.1	55.3	56.5	57.7	59.0	60.2	61.4	62.6	63.8	
9	38.1	39.3	40.5	41.6	42.8	44.0	45.2	46.4	47.6	48.8	50.0	51.2	52.4	53.6	54.8	56.0	57.2	58.4	59.7	60.9	62.1	63.4	64.6	65.8	
10	39.7	40.9	42.1	43.3	44.5	45.7	46.9	48.1	49.3	50.5	51.8	53.0	54.2	55.5	56.7	57.9	59.2	60.4	61.7	62.9	64.2	65.4	66.7	67.9	
11	41.4	42.6	43.8	45.0	46.3	47.5	48.7	49.9	51.2	52.4	53.7	54.9	56.1	57.4	58.6	59.9	61.2	62.4	63.7	65.0	66.3	67.5	68.8	70.1	
12	43.1	44.3	45.6	46.8	48.1	49.3	50.6	51.8	53.1	54.3	55.6	56.8	58.1	59.4	60.7	62.0	63.2	64.5	65.8	67.1	68.4	69.7	71.0	72.4	
13	44.9	46.1	47.4	48.6	49.9	51.2	52.5	53.7	55.0	56.3	57.6	58.9	60.2	61.5	62.8	64.1	65.4	66.7	68.0	69.3	70.7	72.0	73.3	74.7	
14	46.7	48.0	49.3	50.5	51.8	53.1	54.4	55.7	57.0	58.3	59.6	60.9	62.3	63.6	64.9	66.3	67.6	68.9	70.3	71.6	73.0	74.4	75.7	77.1	
15	48.6	49.9	51.2	52.5	53.8	55.1	56.4	57.8	59.1	60.4	61.8	63.1	64.5	65.8	67.2	68.5	69.9	71.3	72.6	74.0	75.4	76.8	78.2	79.6	
16	50.6	51.8	53.2	54.5	55.8	57.2	58.5	59.9	61.2	62.6	64.0	65.3	66.7	68.1	69.5	70.9	72.3	73.7	75.1	76.5	77.9	79.3	80.8	82.2	
17	52.5	53.9	55.2	56.6	58.0	59.3	60.7	62.1	63.5	64.9	66.3	67.7	69.1	70.5	71.9	73.3	74.8	76.2	77.6	79.1	80.5	82.0	83.5	84.9	
18	54.6	56.0	57.4	58.8	60.2	61.6	63.0	64.4	65.8	67.2	68.6	70.1	71.5	73.0	74.4	75.9	77.3	78.8	80.3	81.8	83.3	84.8	86.3	87.8	
19	56.8	58.2	59.6	61.0	62.4	63.9	65.3	66.8	68.2	69.7	71.1	72.6	74.1	75.6	77.0	78.5	80.0	81.5	83.1	84.6	86.1	87.6	89.2	90.7	
20	59.0	60.4	61.9	63.3	64.8	66.3	67.7	69.2	70.7	72.2	73.7	75.2	76.7	78.2	79.8	81.3	82.8	84.4	85.9	87.5	89.1	90.7	92.2	93.8	
21	61.3	62.8	64.3	65.8	67.3	68.8	70.3	71.8	73.3	74.9	76.4	77.9	79.5	81.1	82.6	84.2	85.8	87.4	89.0	90.6	92.2	93.8	95.4	97.1	
22	63.8	65.3	66.8	68.3	69.8	71.4	72.9	74.5	76.1	77.6	79.2	80.8	82.4	84.0	85.6	87.2	88.9	90.5	92.1	93.8	95.5	97.1	98.8	100.5	
23	66.3	67.8	69.4	71.0	72.5	74.1	75.7	77.3	78.9	80.5	82.2	83.8	85.4	87.1	88.7	90.4	92.1	93.8	95.5	97.2	98.9	100.6	102.4	104.1	
24	68.9	70.8	72.1	73.7	75.3	77.0	78.6	80.3	81.9	83.6	85.2	86.9	88.6	90.3	92.0	93.8	95.5	97.2	99.0	100.7	102.5	104.3	106.1	107.9	
25	71.7	73.3	75.0	76.6	78.3	80.0	81.7	83.3	85.1	86.8	88.5	90.2	92.0	93.7	95.5	97.3	99.1	100.9	102.7	104.5	106.3	108.2	110.0	111.9	
26	74.6	76.3	78.0	79.7	81.4	83.1	84.8	86.6	88.4	90.1	91.9	93.7	95.5	97.3	99.2	101.0	102.9	104.7	106.6	108.5	110.4	112.3	114.2	116.2	
27	77.8	79.4	81.1	82.9	84.6	86.4	88.2	90.0	91.9	93.7	95.6	97.4	99.3	101.2	103.1	105.0	106.9	108.8	110.8	112.7	114.7	116.7	118.7	120.7	
28	80.8	82.6	84.4	86.2	88.1	89.9	91.8	93.7	95.6	97.5	99.4	101.3	103.3	105.2	107.2	109.2	111.2	113.2	115.2	117.3	119.3	121.4	123.5	125.6	
29	84.2	86.1	87.9	89.8	91.7	93.7	95.6	97.5	99.5	101.5	103.5	105.5	107.5	109.5	111.6	113.7	115.7	117.8	120.0	122.1	124.2	126.4	128.6	130.8	
30	87.8	89.7	91.7	93.6	95.6	97.6	99.6	101.5	103.7	105.7	107.8	109.9	112.0	114.2	116.3	118.5	120.6	122.8	125.1	127.3	129.5	131.8	134.1	136.4	
31	91.6	93.6	95.6	97.7	99.7	101.8	103.9	106.0	108.2	110.3	112.5	114.7	116.9	119.1	121.4	123.6	125.9	128.2	130.5	132.9	135.3	137.7	140.1	142.5	
32	95.7	97.8	99.9	102.0	104.2	106.3	108.5	110.7	113.0	115.2	117.5	119.8	122.1	124.5	126.8	129.2	131.6	134.0	136.5	139.0	141.5	144.0	146.6	149.1	
33	100.0	102.2	104.4	106.6	108.9	111.2	113.5	115.8	118.2	120.5	122.9	125.4	127.8	130.3	132.8	135.3	137.8	140.4	143.0	145.6	148.3	150.9	153.7	156.4	
34	104.7	107.0	109.3	111.7	114.0	116.4	118.9	121.3	123.8	126.3	128.8	131.4	134.0	136.6	139.2	141.9	144.6	147.4	150.1	152.9	155.7	158.6	161.5	164.4	
35	109.7	112.2	114.6	117.1	119.6	122.2	124.7	127.3	129.9	132.6	135.3	138.0	140.8	143.6	146.4	149.2	152.1	155.0	158.0	161.0	164.0	167.1	170.2	173.3	
36	115.2	117.8	120.4	123.0	125.7	128.4	131.1	133.9	136.7	139.5	142.4	145.3	148.3	151.3	154.3	157.3	160.5	163.6	166.8	170.0	173.3	176.6	179.9	183.3	
37	121.3	124.0	126.8	129.6	132.4	135.3	138.2	141.2	144.2	147.3	150.3	153.5	156.7	159.9	163.1	166.5	169.8	173.2	176.7	180.2	183.7	187.3	191.0	194.7	
38	127.9	130.8	133.8	136.8	139.9	143.0	146.2	149.4	152.6	155.9	159.2	162.6	166.1	169.5	173.2	176.8	180.4	184.2	188.0	191.8	195.7	199.7	203.7	207.7	
39	135.3	138.5	141.7	145.0	148.3	151.7	155.1	158.6	162.1	165.7	169.4	173.1	176.9	180.7	184.7	188.7	192.7	196.8	201.0	205.3	209.6	214.0	218.5	223.0	
40	143.7	147.1	150.6	154.2	157.8	161.5	165.3	169.1	173.0	177.0	181.1	185.2	189.4	193.7	198.1	202.5	207.1	211.7	216.4	221.1	226.0	231.0	236.0	241.1	
41	153.2	157.0	160.9	164.8	168.9	173.0	177.2	181.5	185.8	190.3	194.8	199.5	204.2	209.1	214.0	219.1	224.2	229.4	234.8	240.2	245.8	251.5	257.2	263.1	
42	164.3	168.6	172.9	177.3	181.9	186.5	191.3	196.1	201.1	206.2	211.4	216.7	222.2	227.7	233.4	239.2	245.2	251.3	257.5	263.8	270.3	276.9	283.6	290.5	
43	177.5	182.3	187.3	192.4	197.6	202.9	208.4	214.0	219.8	225.8	231.8	238.1	244.5	251.0	257.7	264.6	271.7	278.9	286.3	293.8	301.5	309.4	317.4	325.7	
44	193.6	199.3	205.1	211.0	217.2	223.5	230.0	236.7	243.6	250.8	258.1	265.6	273.3	281.2	289.4	297.8	306.3	315.1	324.1	333.3	342.8	352.4	362.3	372.4	
45	214.1	220.9	227.9	235.2	242.7	250.4	258.4	266.7	275.3	284.1	293.3	302.6	312.3	322.3	332.5	343.0	353.8	364.9	376.2	387.9	399.8	412.0	424.5	437.4	
46	241.5	250.0	258.9	268.2	277.8	287.8	298.1	308.8	319.9	331.4	343.3	355.5	368.1	381.1	394.5	408.3	422.5	437.1	452.0	467.4	483.3	499.6	516.3	533.5	
47	280.9	292.4	304.4	316.9	330.0	343.6	357.8	372.5	387.7	403.4	419.8	436.6	454.1	472.1	490.7	509.9	529.8	550.4	571.7	593.8	616.7	640.5	665.3	691.0	
48	344.1	360.9	378.4	396.8	416.0	436.0	456.9	478.8	501.2	524.7	549.3	574.8	601.5	629.4	658.6	689.3	721.5	755.6	791.5	829.7	870.4	913.9	960.6	1011.2	
49	461.1	488.4	517.2	547.5	579.4	613.1	648.8	686.7	727.0	770.1	816.4	866.4	920.8	980.4	1046.2	1119.9	1203.3	1299.7	1413.6	1553.1	1732.9	1998.3	2419.6	>2419.6	

09-63236-01

Total Coliform/E. coli by Quanti-Tray®

MPN table for Quanti-Tray®

IDEXX
51-Well Quanti-Tray®
MPN Table

No. of wells giving positive reaction	MPN per 100 ml sample	95% Confidence Limits Lower	Upper
0	<1.0	0.0	3.7
1	1.0	0.3	5.6
2	2.0	0.6	7.3
3	3.1	1.1	9.0
4	4.2	1.7	10.7
5	5.3	2.3	12.3
6	6.4	3.0	13.9
7	7.5	3.7	15.5
8	8.7	4.5	17.1
9	9.9	5.3	18.8
10	11.1	6.1	20.5
11	12.4	7.0	22.1
12	13.7	7.9	23.9
13	15.0	8.8	25.7
14	16.4	9.8	27.5
15	17.8	10.8	29.4
16	19.2	11.9	31.3
17	20.7	13.0	33.3
18	22.2	14.1	35.2
19	23.8	15.3	37.3
20	25.4	16.5	39.4
21	27.1	17.7	41.6
22	28.8	19.0	43.9
23	30.6	20.4	46.3
24	32.4	21.8	48.7
25	34.4	23.3	51.2
26	36.4	24.7	53.9
27	38.4	26.4	56.6
28	40.6	28.0	59.5
29	42.9	29.7	62.5
30	45.3	31.5	65.6
31	47.8	33.4	69.0
32	50.4	35.4	72.5
33	53.1	37.5	76.2
34	56.0	39.7	80.1
35	59.1	42.0	84.4
36	62.4	44.6	88.8
37	65.9	47.2	93.7
38	69.7	50.0	99.0
39	73.8	53.1	104.8
40	78.2	56.4	111.2
41	83.1	59.9	118.3
42	88.5	63.9	126.2
43	94.5	68.2	135.4
44	101.3	73.1	146.0
45	109.1	78.6	158.7
46	118.4	85.0	174.5
47	129.8	92.7	195.0
48	144.5	102.3	224.1
49	165.2	115.2	272.2
50	200.5	135.8	387.6
51	> 200.5	146.1	infinite

IDEXX Sales and Technical Support
1-800-321-0207 or 1-207-856-0496
www.idexx.com/water

09-63234-00

Total Coliform/E. coli by Quanti-Tray®

Questions?

Thank you for your attention! Questions?

For more information, please contact:

John Allen,

John.allen@fairfaxcounty.gov

ROLE AND RELATIONSHIP OF MICROORGANISM IN WASTEWATER, THE WASTEWATER TREATMENT PLANT AND TESTING OF *e. COLI IN THE npdes* PERMIT

Saiful Islam
Industrial Waste Section
John Allen
Environmental Monitoring Branch

PRESENTATION SETUP

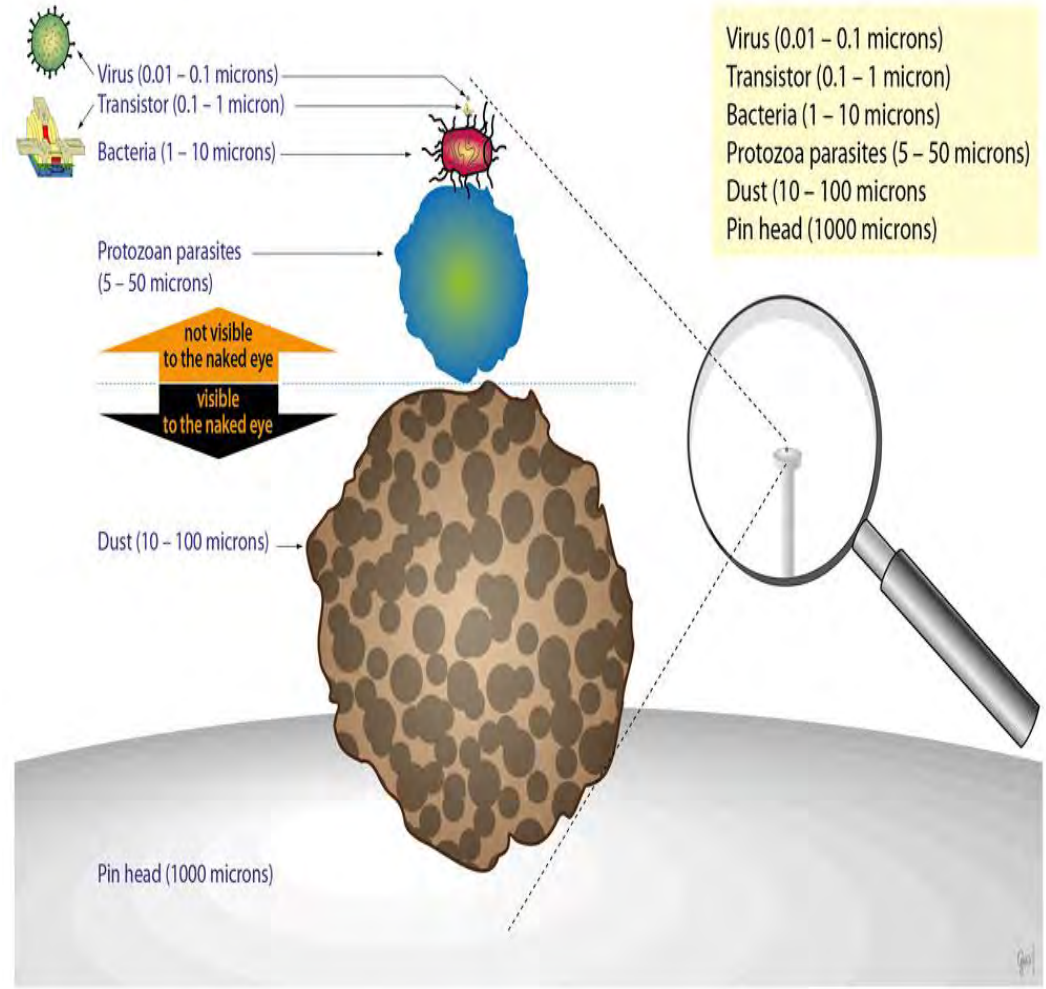
1. What are microbes- Slide 3

- 2. The wastewater Treatment Plant-
- Industrial Waste section, Noman M. Cole Pollution Control Plant and its process, and the biology of microbes Slide 9

- 3. Microbiology- Water Quality Testing- Indicator Organism- Slide 48

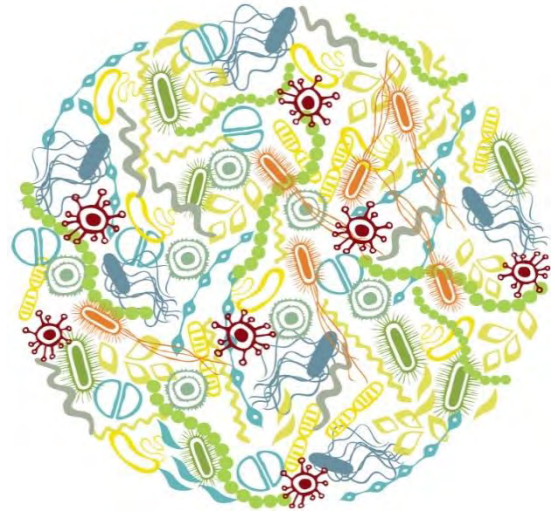
1. WHAT ARE MICROBES?

- A microbe, or "microscopic organism," is a living thing that is too small to be seen with the naked eye.
- The term is very general.



WHAT ARE MICROBES?

- Microbes comes in different sizes and characteristics:
- Bacteria
- Archaea
- Fungi
- Protists
- Viruses
- Microscopic animals

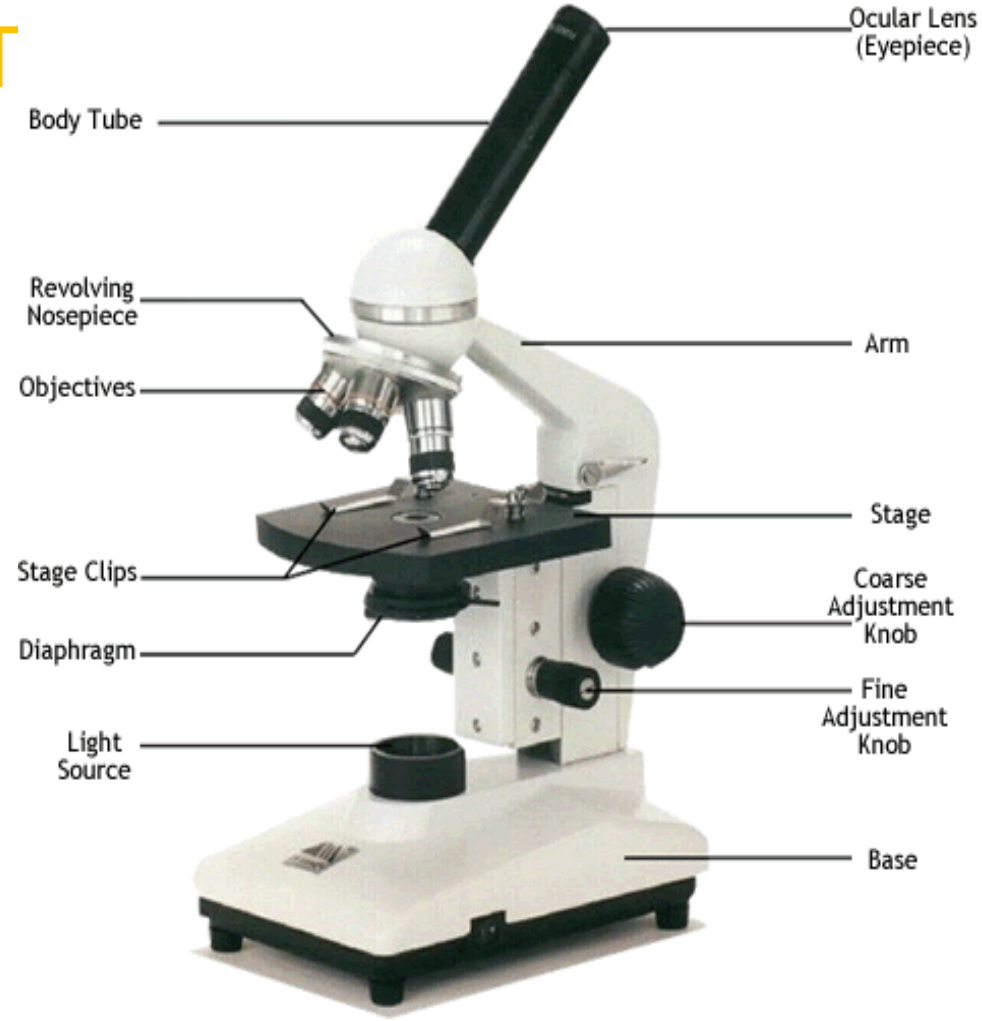


- The human body is home to microbes from all of these categories.
-

http://learn.genetics.utah.edu/content/microbiome/intro/#bacteria

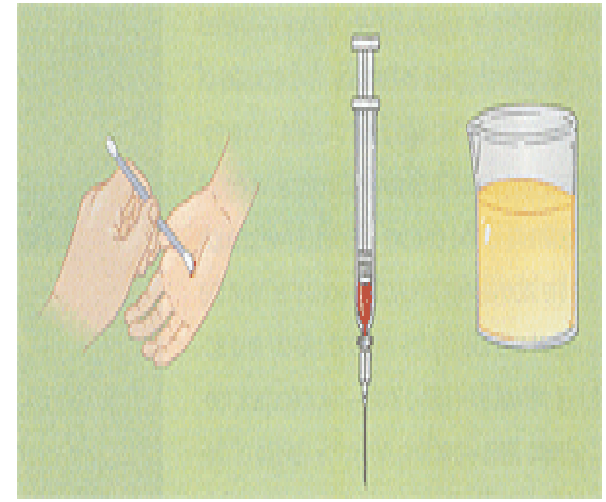
The Microscope

- The principal way a microbiologist studies microorganisms is by observing them through a microscope.
- A **microscope** is a device that enlarges objects using a process called magnification.



THE FIVE I'S

- The Five I's is a method used to locate, grow, observe and characterize microorganisms.
- The first step is the collect your specimen.
- Common specimens are body fluids, foods, water or soil.

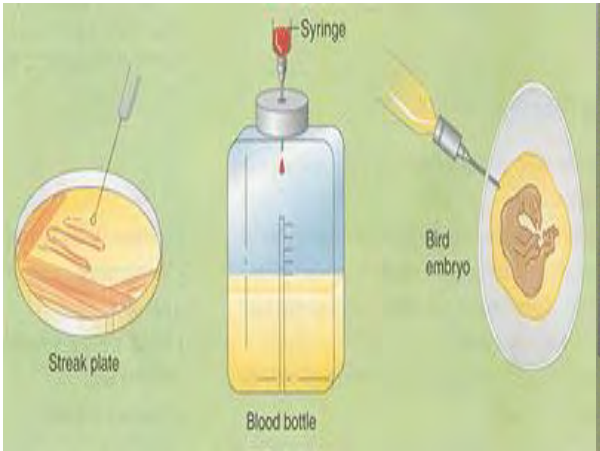


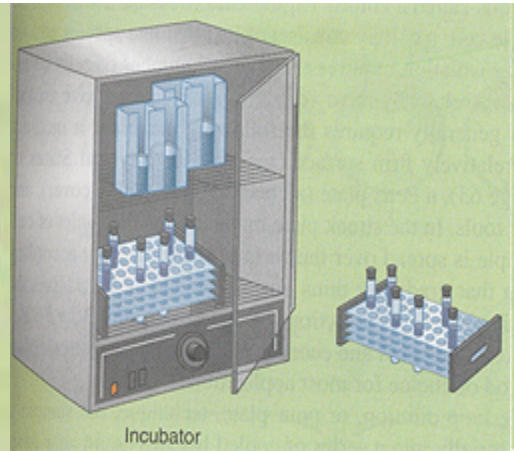
THE FIVE I'S

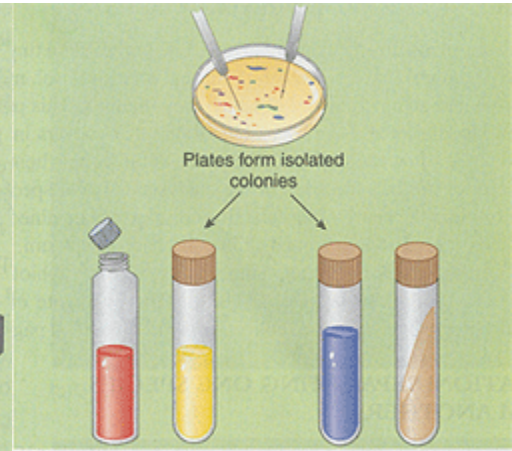
- One you have your specimen collected you can perform the Five I's:

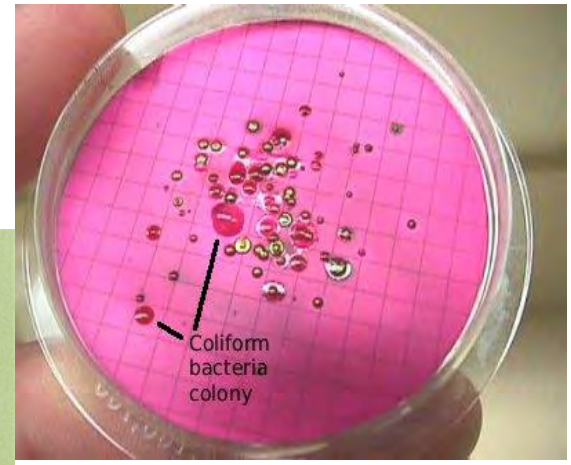
1. Inoculation: The sample is placed into a container of sterile medium that provides microbes with the appropriate nutrients to sustain growth.
2. Incubation: An incubator can be used to adjust the proper growth conditions of a sample.
3. Isolation: The end result of inoculation and incubation is isolation of the microbe.

•1. 2. 3.







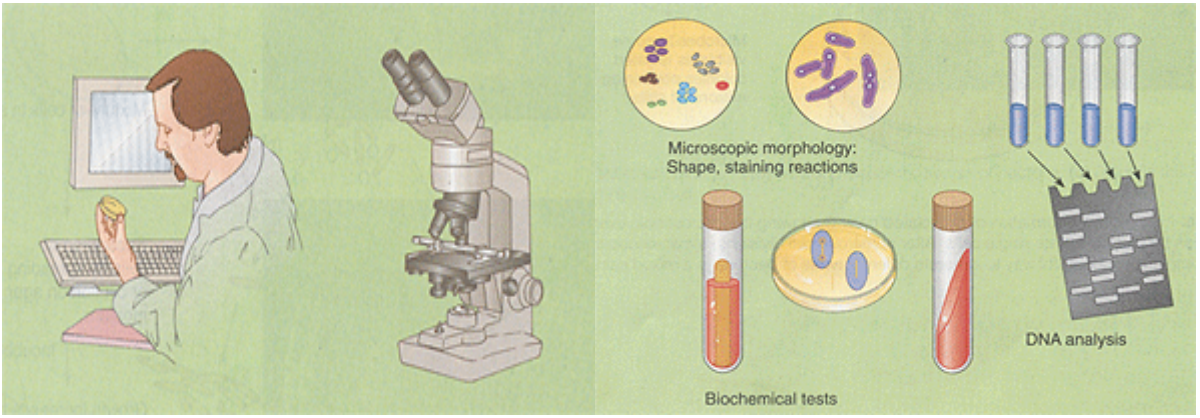


THE FIVE I'S

• 4. Inspection: The cultures are observed for obvious growth characteristics that could be useful in analyzing the specimen contents.

5. Identification: Determine the type of microbe, usually to the level of species.

•Why to identify and what is the benefit of microscopic examination????

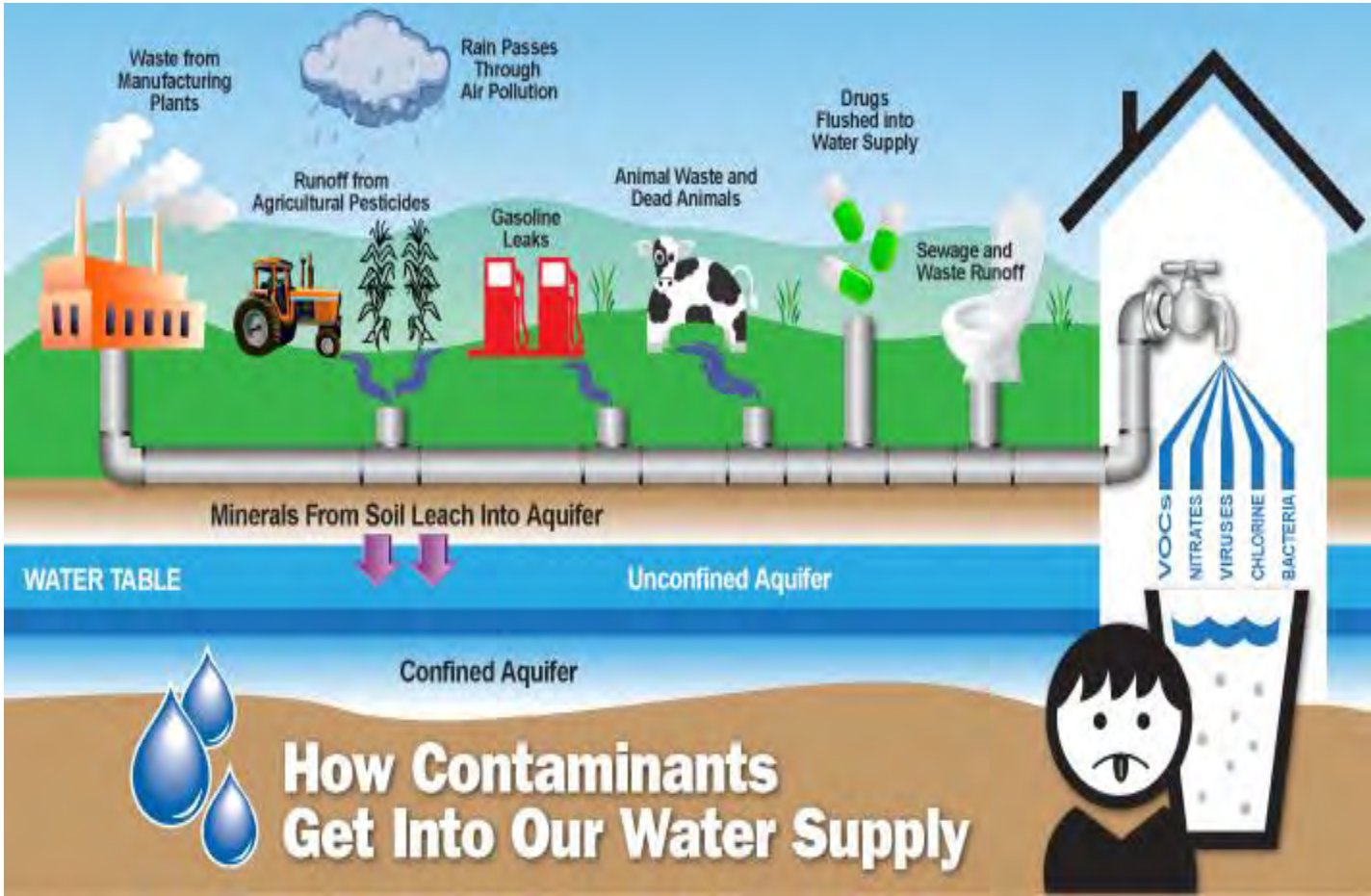


2. WASTEWATER TREATMENT???

- Wastewater treatment practice- 20th century
- Mainly- increased awareness of role of microbes in disease
- Urbanization- population density
- Rivers ran as sewers- epidemics- cholera

WASTEWATER TREATMENT PLANT CONCEPT

- WHY?
- **<u>The principal source/cause of waterborne disease</u>**
- Contamination of water supplies by untreated or improperly treated sewage.



WASTEWATER TREATMENT OBJECTIVE

- Removal/inactivation of pathogenic microbes and parasites
- Reduction of organic content
- Removal/reduction of toxic metals
- Removal/reduction of nutrients (N,P)
- Preserve natural resources- example-eutrophication

Noman M. Cole Pollution Control Plant

- An award-winning wastewater treatment plant
- Owned and operated by Fairfax County Government
- Located in Lorton, Va.

NOMAN M. COLE POLLUTION CONTROL PLANT

- A permitted facility
- VPDES (Virginia Pollutant Discharge Elimination System) Permit

COMMONWEATH OF VIRGINIA
DEPARTMENT OF ENVIRONMENTAL QUALITY

2/5/14

Additional Reporting for Reclamation Systems Authorized by or in Association with a VPDES Permit

NAME Noman M. Cole, Jr. Pollution Control Plant
ADDRESS P. O. Box 268
Lorton, VA 22079
FACILITY
LOCATION 9399 Richmond Hwy

VA0025364	650
PERMIT NUMBER	DISCHARGE NUMBER
MONITORING PERIOD	

DEPT. OF ENVIRONMENTAL QUALITY
(REGIONAL OFFICE)
Northern Regional Office
1309 Crown Court
Woodbridge, VA 22193

NPDES Monitoring Requirements

- The Environmental Protection Agency (EPA) mandates
- Any discharge to a body of water must be permitted through the National Pollutant Discharge Elimination System (NPDES).
- Delegated to Virginia DEQ

NOMAN M. COLE POLLUTION CONTROL PLANT

- Is an approved permit
- Contains effluent limitations
- Monitoring requirements.

Permit Requirement- Monitoring

Parameter	Discharge Limitations				Monitoring Requirements	
	Monthly Average[1]	Weekly Average[1]	Minimum	Maximum[1]	Frequency	Sample Type
Flow[2] (MGD)	NL	NA	NA	NL	Continuous	TIRE
pH	NA	NA	6.0 S.U.	9.0 S.U.	1/D	Grab
$cBOD_5$	5 mg/L 1268 kg/day	8 mg/L 2029 kg/day	NA	NA	3D/W[3]	24H-C
Total Suspended Solids, TSS	6.0 mg/L 1522 kg/day	9.0 mg/L 2282 kg/day	NA	NA	1/D	24H-C
Ammonia as Nitrogen (April-Oct)	1.0 mg/L 254 kg/day	1.5 mg/L 380 kg/day	NA	NA	1/D	24H-C
Ammonia as Nitrogen (Nov-March)					1/D	24H-C
Dissolved Oxygen					1/D	Grab
Total Residual Chlorine (after contact tank)					12/D at 2 hr intervals	Grab
Total Residual Chlorine (after dechlorination)	0.008 mg/L	0.010 mg/L	NA	NA	12/D at 2 hr intervals	Grab
E. coli (Geometric Mean)	126 n/100 mls	NA	NA	NA	5D/W[4]	Grab
Total Nitrogen[5] – Monthly (mg/L)	NL	NA	NA	NA	3D/W	Calculated
Total Kjeldahl Nitrogen, TKN (mg/L)	NL	NA	NA	NA	3D/W	24H-C
Nitrate+Nitrite as Nitrogen (mg/L)	NL	NA	NA	NA	3D/W	24H-C
Total Nitrogen – Calendar Year[6]	7.0 mg/L	NA	NA	NA	1/YR	Calculated
Total Nitrogen – Year-To-Date[6](mg/L)	NL	NA	NA	NA	1/M	Calculated
Total Phosphorus	0.18 mg/L 46.6 kg/d	0.27 mg/L 68.5 kg/d	NA	NA	1/D	24H-C
Chronic 3-Brood Static Renewal– *C. dubia* (TU_c)[7]	NA	NA	NA	NL	1/YR	24H-C
Chronic 7-Day Static Renewal - *P. promelas* (TU_c)[7]	NA	NA	NA	NL	1/YR	24H-C

A. Effluent Limitations and Monitoring Requirements

1. Outfall 001- 67 MGD Facility

HH building- Effluent Sampling site for E. *Coli*

NOMAN M. COLE POLLUTION CONTROL PLANT

- Also required by NPDES permit
- The pretreatment requirements

C. Pretreatment Requirements

1. The permittee's pretreatment program has been approved. The program is an enforceable part of this permit. The permittee shall:

The Industrial Waste Section (IWS) implements the National Pretreatment Program within Fairfax County

C. Pretreatment Requirements

1. The permittee's pretreatment program has been approved. The program is an enforceable part of this permit. The permittee shall:

   a. Within 180 days of the effective date of this permit, submit to the DEQ-Northern Regional Office (NRO) a survey of all Industrial Users meeting the requirements of the VPDES Permit regulation, 9VAC25-31-10 et seq. and who is discharging to the POTW. The information shall be submitted to the POTW on the DEQ's Discharger Survey Form or an equivalent form that includes the quantity and quality of the wastewater. Survey results shall include the identification of significant industrial users of the POTW.

      In lieu of the survey, the permittee may elect to develop and submit for approval a plan to continuously survey the industrial community in their jurisdiction. This plan must be implemented within 90 days of its approval by DEQ-NRO.

   b. Within one year of the effective date of this permit, the permittee shall develop or reevaluate the local limits using current influent, effluent and sludge monitoring data and submit the data and results of the evaluation to DEQ-NRO. All Significant Industrial Users shall be sampled at the end of any categorical process and at the entrance to the treatment works.

   c. Submit to the DEQ-NRO an annual report that describes the permittee's program activities over the previous year. The annual report shall be submitted no later than January 31 of each year and shall include:

Industrial Waste/Pretreatment

- The Industrial Waste Section regulates industrial users of the sanitary sewer system in accordance with the Clean Water Act.
- County businesses are surveyed, inspected and monitored
- To prevent toxic or harmful substances from entering the collection system and reaching the treatment plant.


Sampling Wastewater

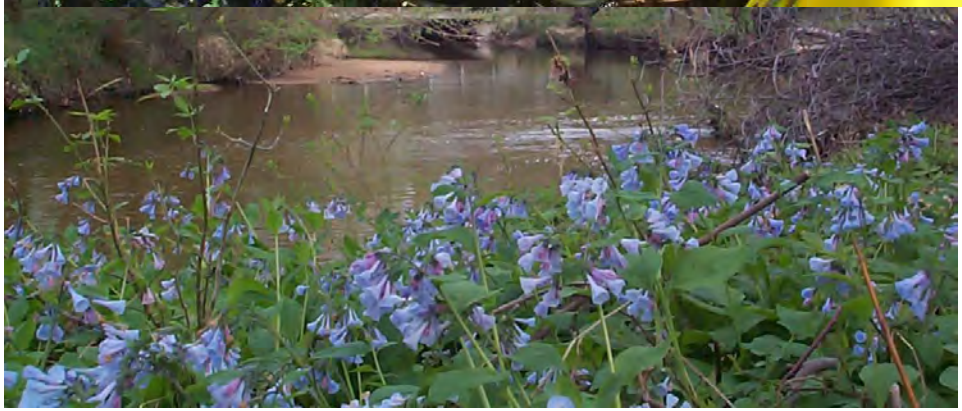

Analyzing


Septage Receiving Site


Sampling Surface Water

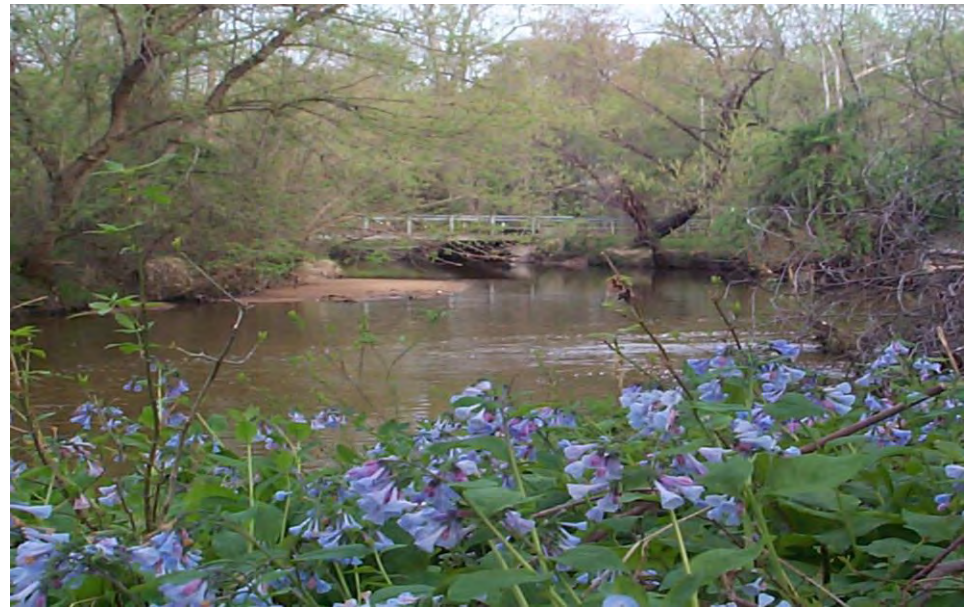
Pollution

- The term-
- The man-made or man induced alteration of the chemical, physical, biological and radiological integrity of water.



Clean water Act Goal

- Is “to restore and maintain the chemical, physical, and biological integrity of the Nation’s waters”



WASTEWATER TREATMENT PLANT CONCEPT

- **Purpose of treatment plant**
- The treatment of human fecal wastes
- → organic matter plus many bacterial, protozoan & viral pathogens
- One of the most important factors in maintaining an advanced healthy society.

MICROBES AND THE PLANT

- **Microorganisms**
- Principally bacteria
- Metabolize organic material and inorganic ions present in wastewater during growth

VIDEO BACTERIA

MICROBES AND THE PLANT

- Bacteria are classified in two categories depending on their source of energy
- 1. Heterotrophs- use organic matter as a carbon source and as an energy source

MICROBES AND THE PLANT

- Further classified into three groups by their environment they can function in.
- A. Aerobics- decompose organic matter while using free oxygen
- B. Anaerobic- function in absence of oxygen- oxygen is toxic to them
- C. Facultative- use free oxygen when it is available but when it isn't, they function as do the anaerobic types.

COMPOUNDS CONTAINING:	WILL DECAY AEROBICALLY TO:	WILL DECAY ANAEROBICALLY TO:
Carbon (C)	CO_2	CH_4, CO_2
Phosphorous (P)	H_3PO_4 (orthophosphate)	PH_3 (Phosphine)
Nitrogen (N)	NO_3 (NO_x)	NH_3, amines
Sulfur(S)	SO_4 (SO_x)	H_2S

MICROBES AND THE PLANT

- 2. Autotrophs- eg. Iron bacteria obtain energy from the oxidation of ferrous iron to insoluble ferric iron
- They use bicarbonates (HCO_3) as their source of carbon

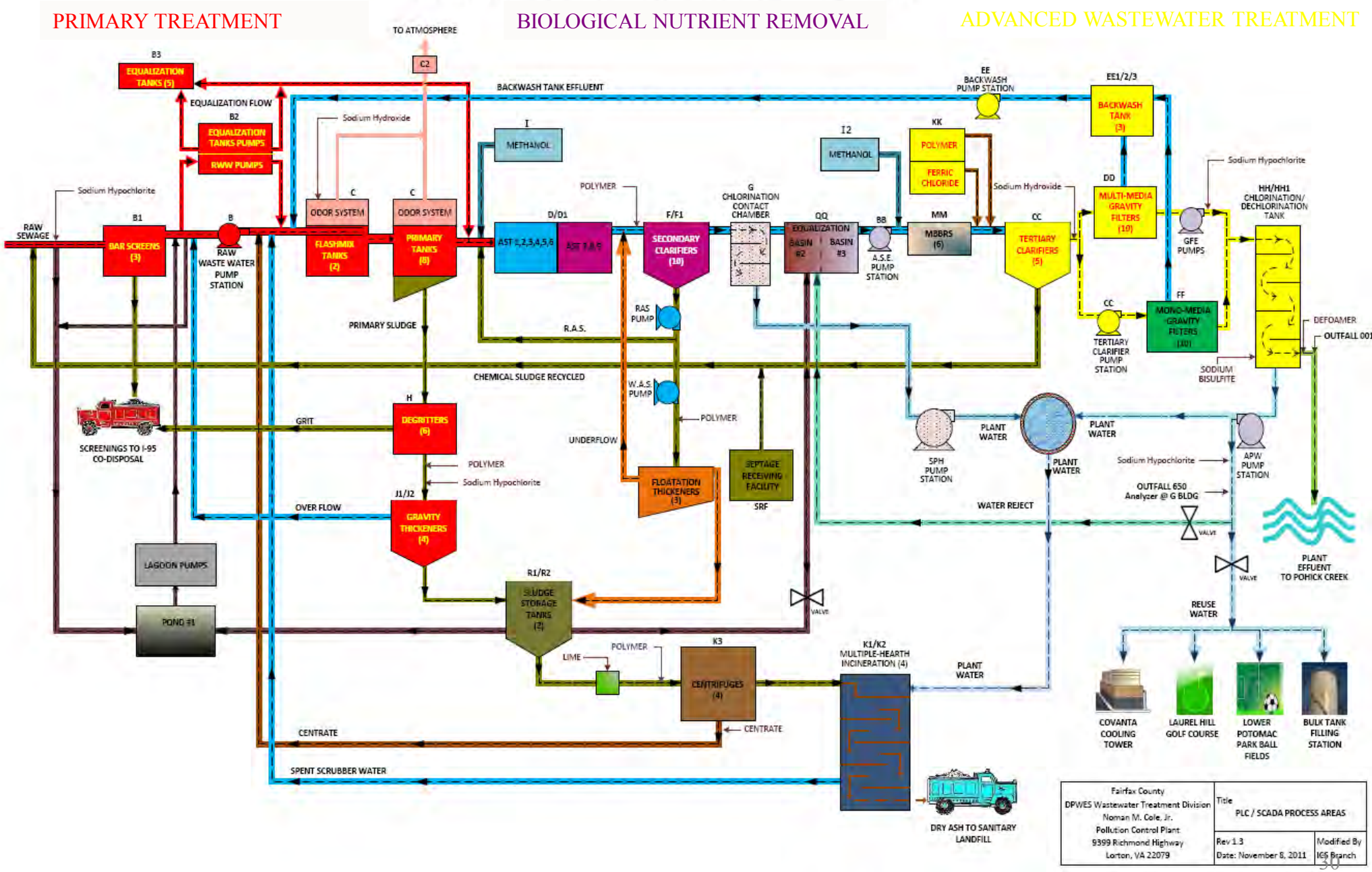
http://www.fairfaxcounty.gov/dpwes/stormwater/stuffinstream.htm

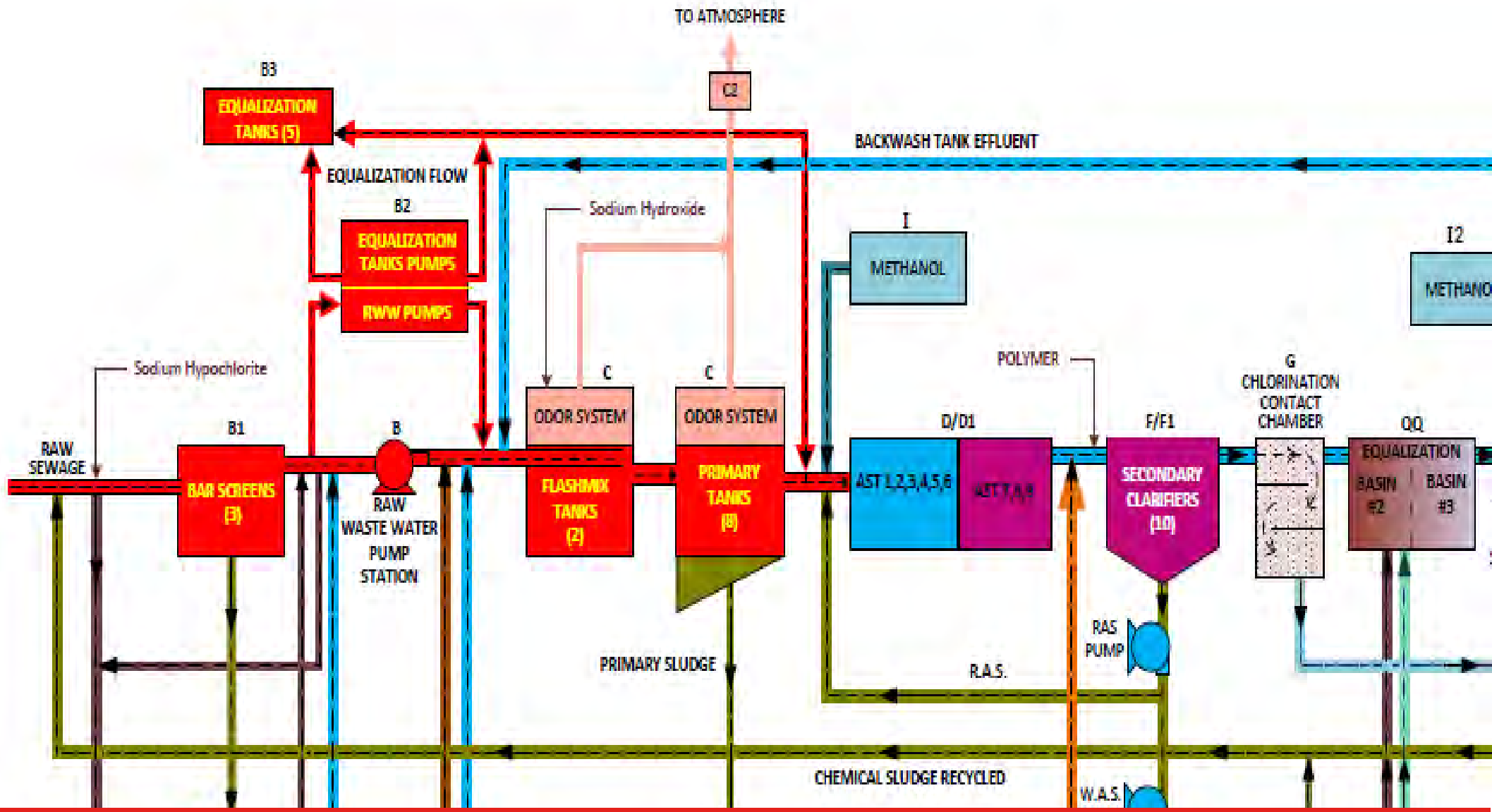
WASTEWATER TREATMENT PLANT

- Conventional sewage treatment is a controlled intensification of natural self-purification processes involving 1°, 2°, and 3° treatmen
- Primary- Physical
- Secondary- Biological
- Tertiary- Chemical

VIDEO WASTEWATER TREATMENT PROCESS

WASTEWATER TREATMENT PROCESS AREAS

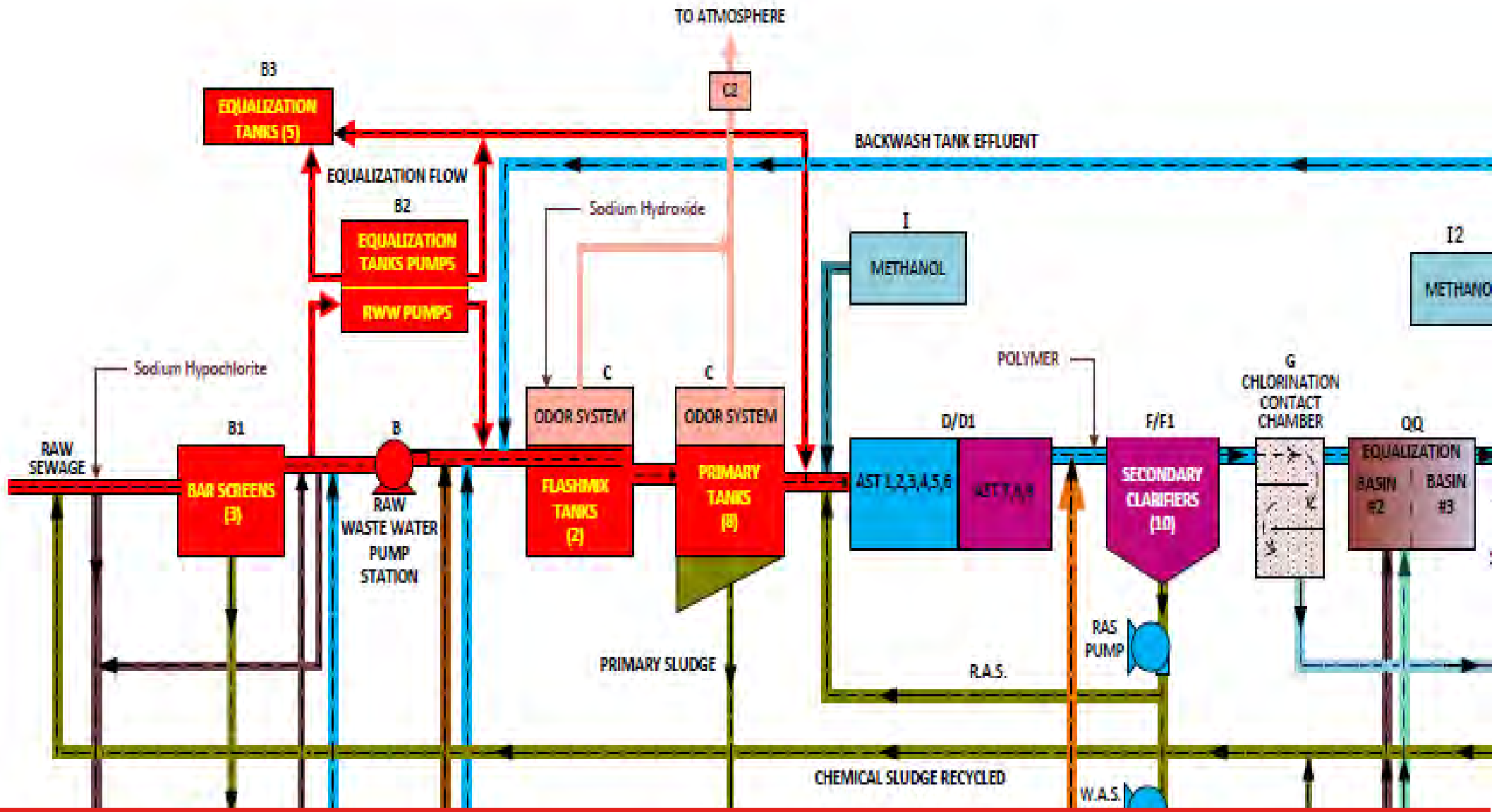




Process- raw sewage → bar screens →Flash mix tank → Primary tank

Removes solids

Waste has high nutrient load (eg C, N, S, and P)



Process- raw sewage → bar screens →Flash mix tank → Primary tank

Removes solids

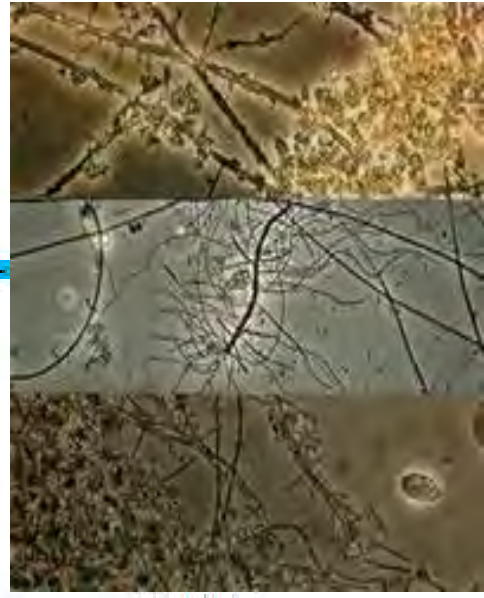
Waste has high nutrient load (eg C, N, S, and P)

- **Secondary treatment**:
- Process- Aeration Tank → Secondary Clarifier

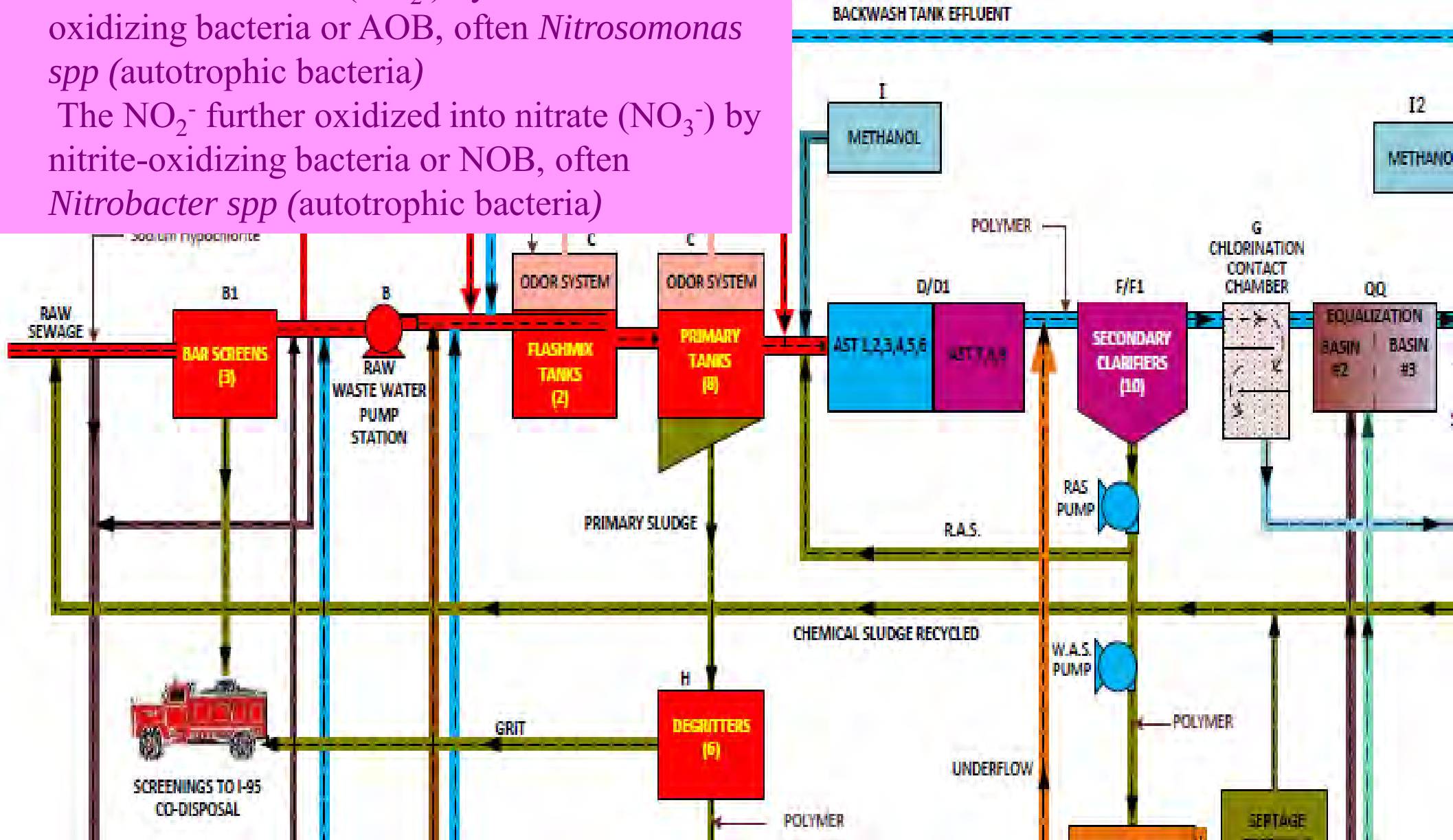
Air bubbled through waste water.

Bacteria form large flocs-

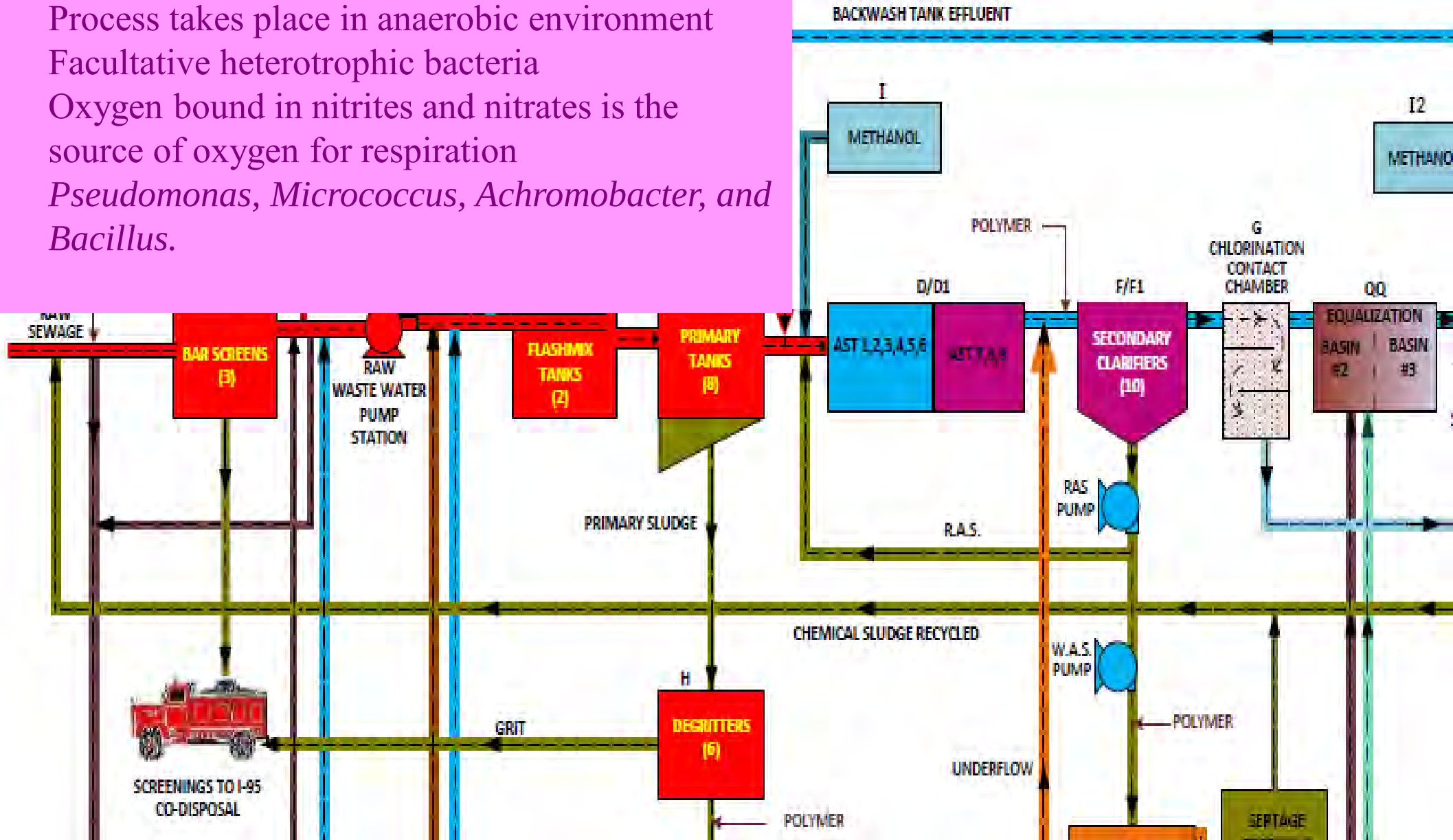
- Microbial conversion of organic matter into microbial biomass
- 90- 95% reduction in BOD
- Removal of many bacterial pathogens.
- Decreases dissolved organic carbon (DOC)
- Aerobic and anaerobic secondary treatment
- VIDEO WASSTEWATER ORGANISMS



- Nitrification is the process
- Ammonium (NH_4^+) or ammonia (NH_3) is oxidized into nitrite (NO_2^-) by ammonia-oxidizing bacteria or AOB, often *Nitrosomonas spp (*autotrophic bacteria*)*
- The NO_2^- further oxidized into nitrate (NO_3^-) by nitrite-oxidizing bacteria or NOB, often *Nitrobacter spp (*autotrophic bacteria*)*



- Denitrifying bacteria- de-nitrification
- Free or reduce nitrates to gaseous nitrogen and some nitrous oxide
- Process takes place in anaerobic environment
- Facultative heterotrophic bacteria
- Oxygen bound in nitrites and nitrates is the source of oxygen for respiration
- *Pseudomonas, Micrococcus, Achromobacter, and Bacillus.*



Video- nutrient removal

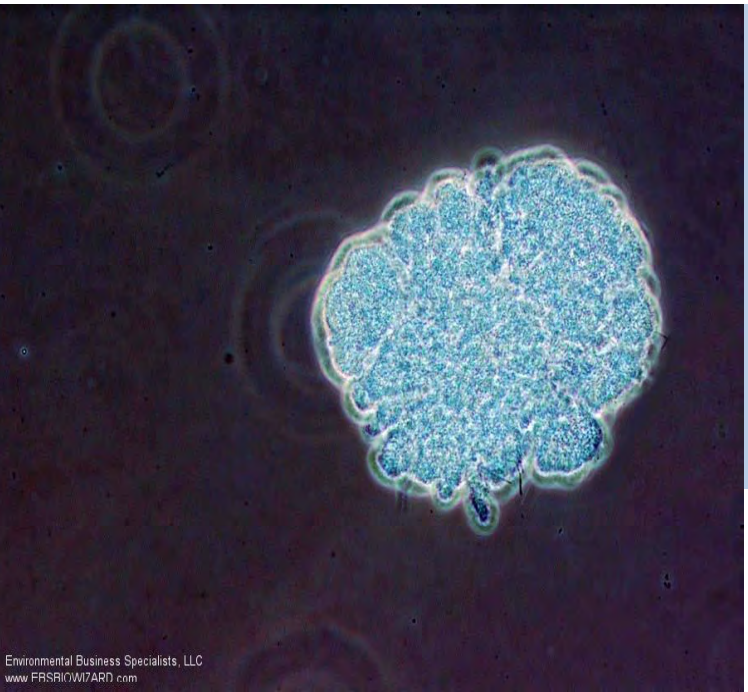
DURATION- 12 MINUTES

Video- wastewater microbiology

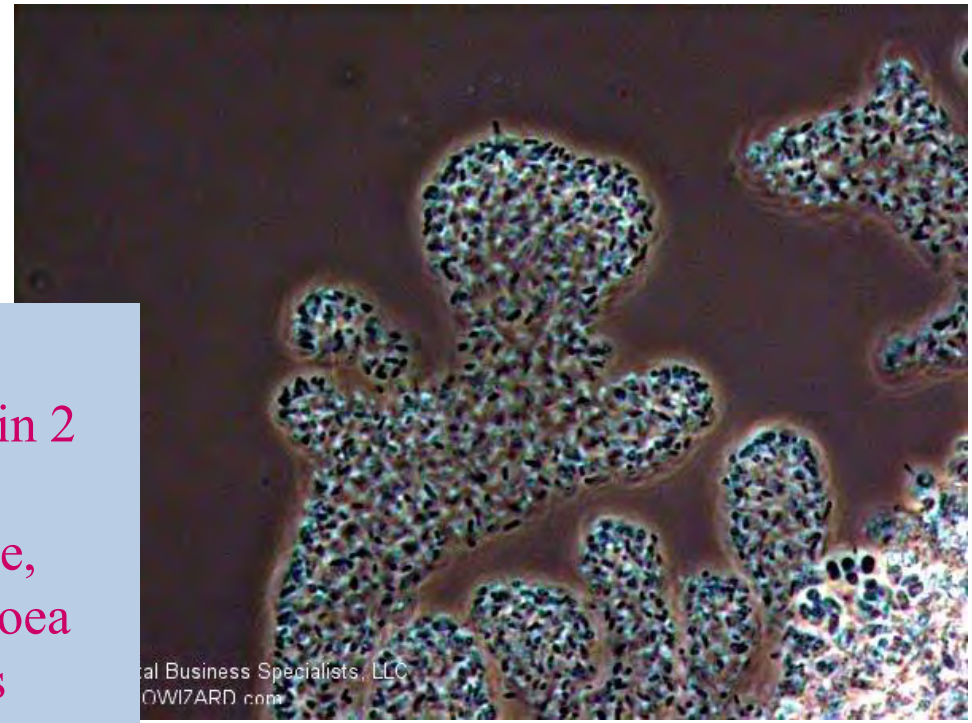
DURATION- 8.36 MINUTES

AEROBIC SECONDARY WASTE TREATENT

- Zoogloea *ramigera* is one of the key species
- Forms a **slime** and is the base of the **floc**.
- After the flocs form they are allowed to settle out



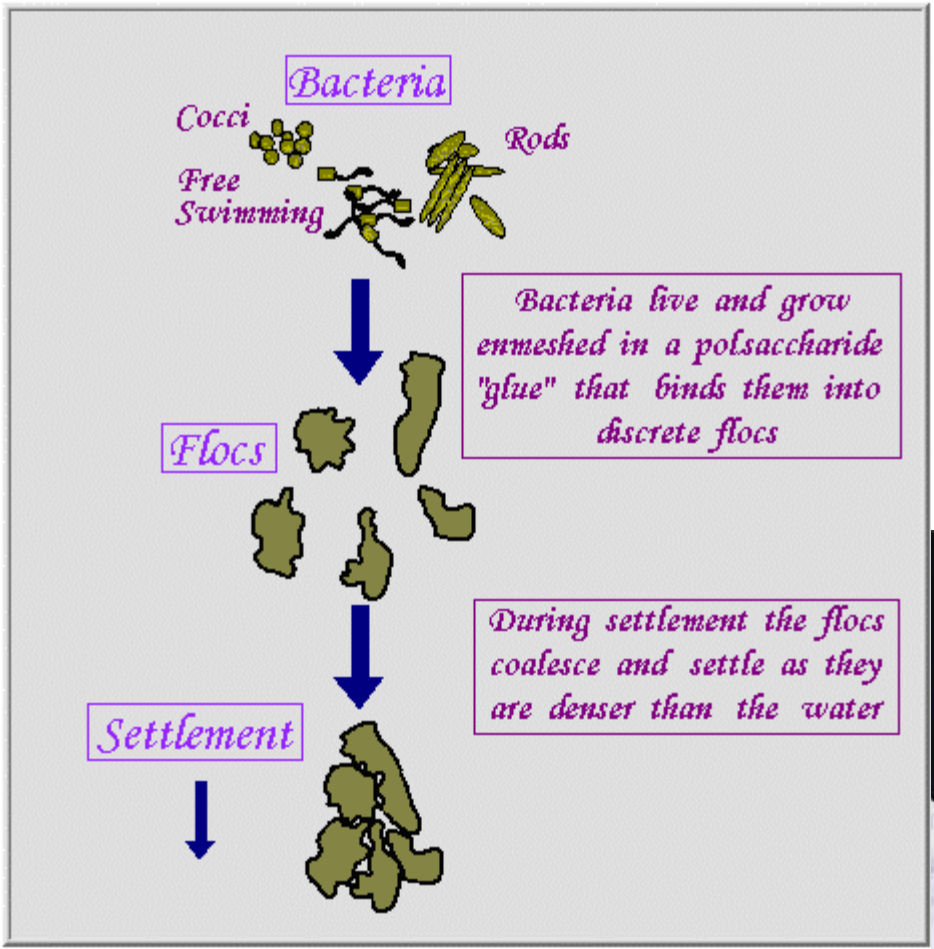
"classical floc-former" exists in 2 forms within activated sludge, fingered Zoogloea and amorphous Zoogloea.

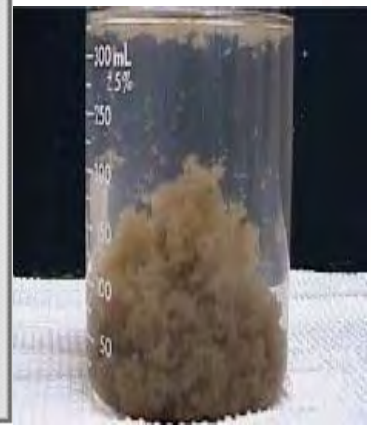


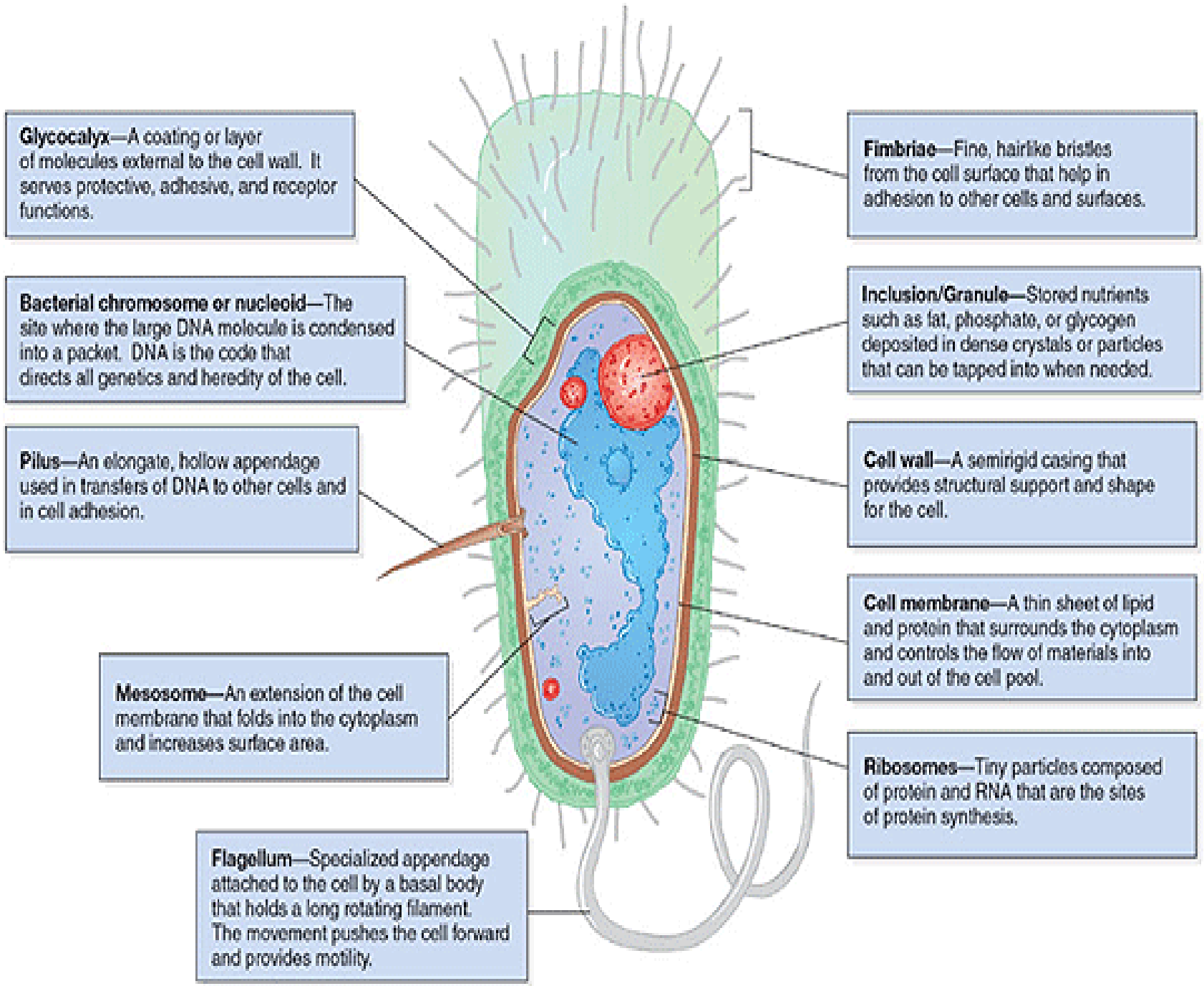
BACTERIA- THE FLOC

http://web.deu.edu.tr/atiksu/ana52/ani409.html

- Floc-forming- formation of an extracellular polysaccharide ("slime") layer- **glycocalyx**.
- Polysaccharide, protein, cellulose fibrils, "cements" the bacteria together to form a floc.







•Glycocalyx (sugar coat)- secretes a substance on their surface
•Surrounds cell
•Viscous (sticky)
•Gelatinous polymer- composed of polysaccharides, polypeptides or both
•Varies with species

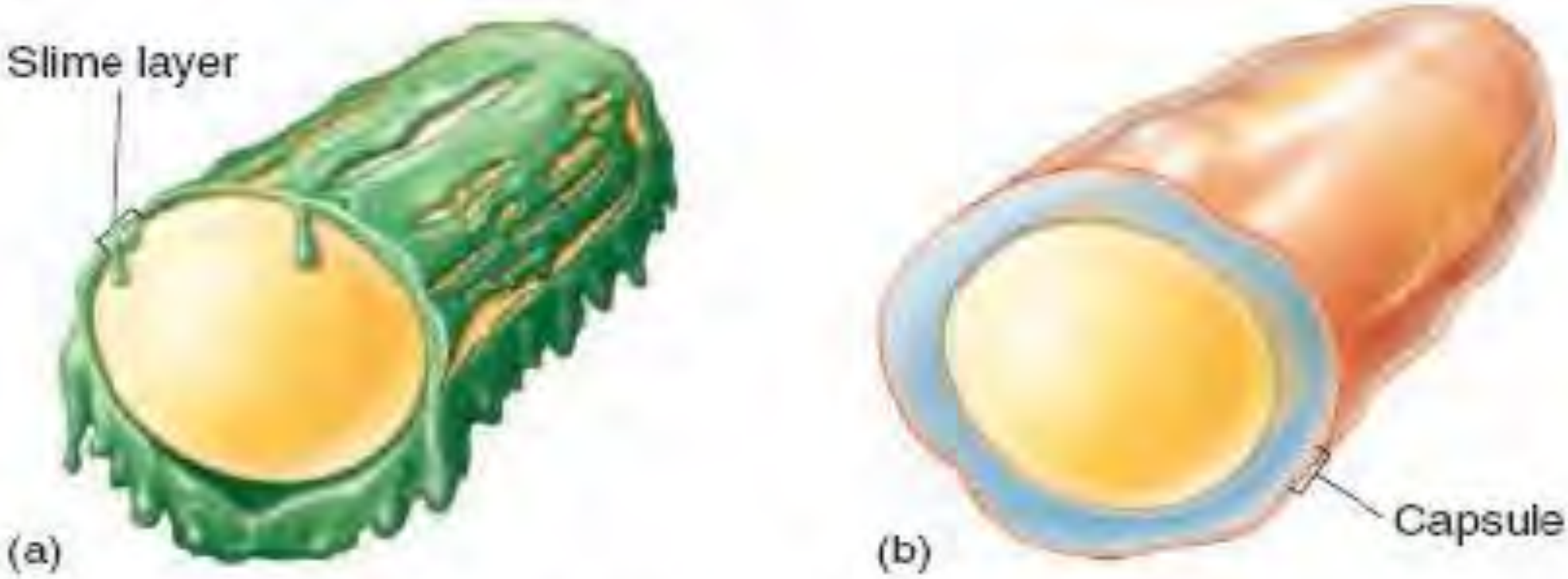


Figure 4.10 Types of glycocalyces seen through cutaway views of cells. (a) The slime layer is a loose structure that is easily washed off. (b) The capsule is a thick, structured layer that is not readily removed.

- Promotes adherence to environmental surfaces
- Development of biofilms
- Serves as receptor and communication function
- Offer some protection against environmental changes

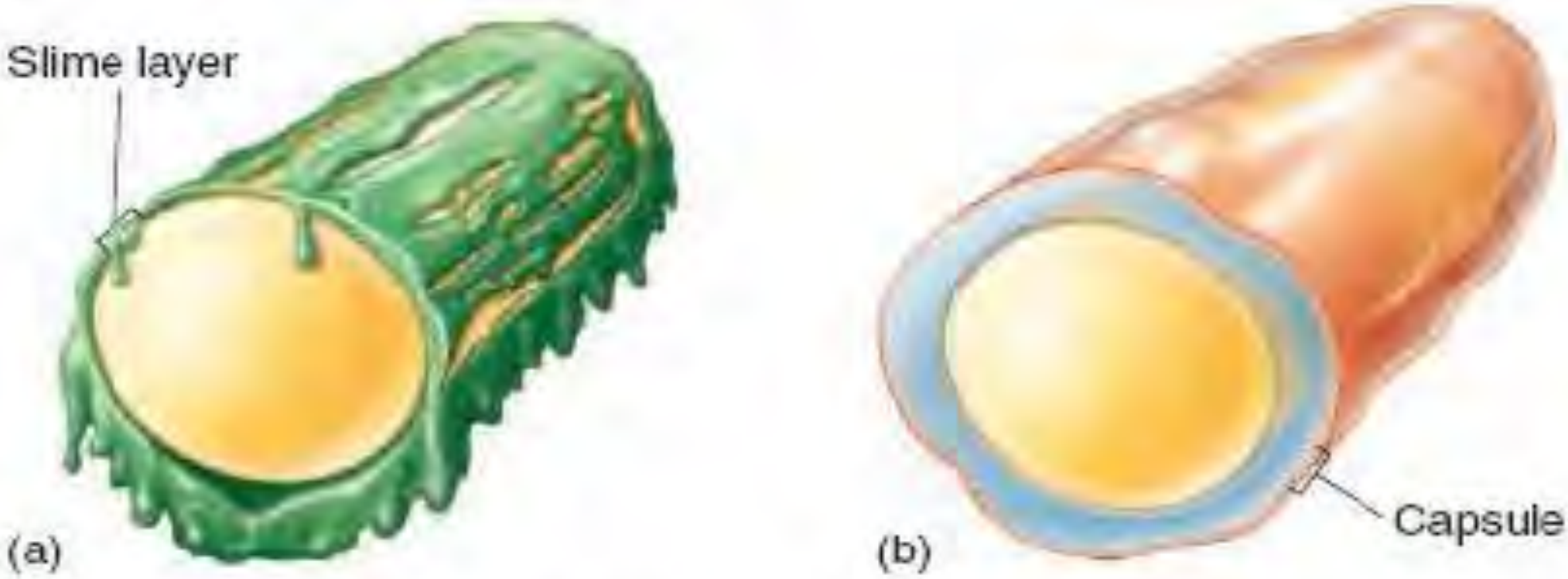


Figure 4.10 Types of glycocalyces seen through cutaway views of cells. (a) The slime layer is a loose structure that is easily washed off. (b) The capsule is a thick, structured layer that is not readily removed.

- Substance is unorganized
- Loosely attached to cell wall

•CAPSULE:
•Substance is organized
•Firmly attached to cell wall-

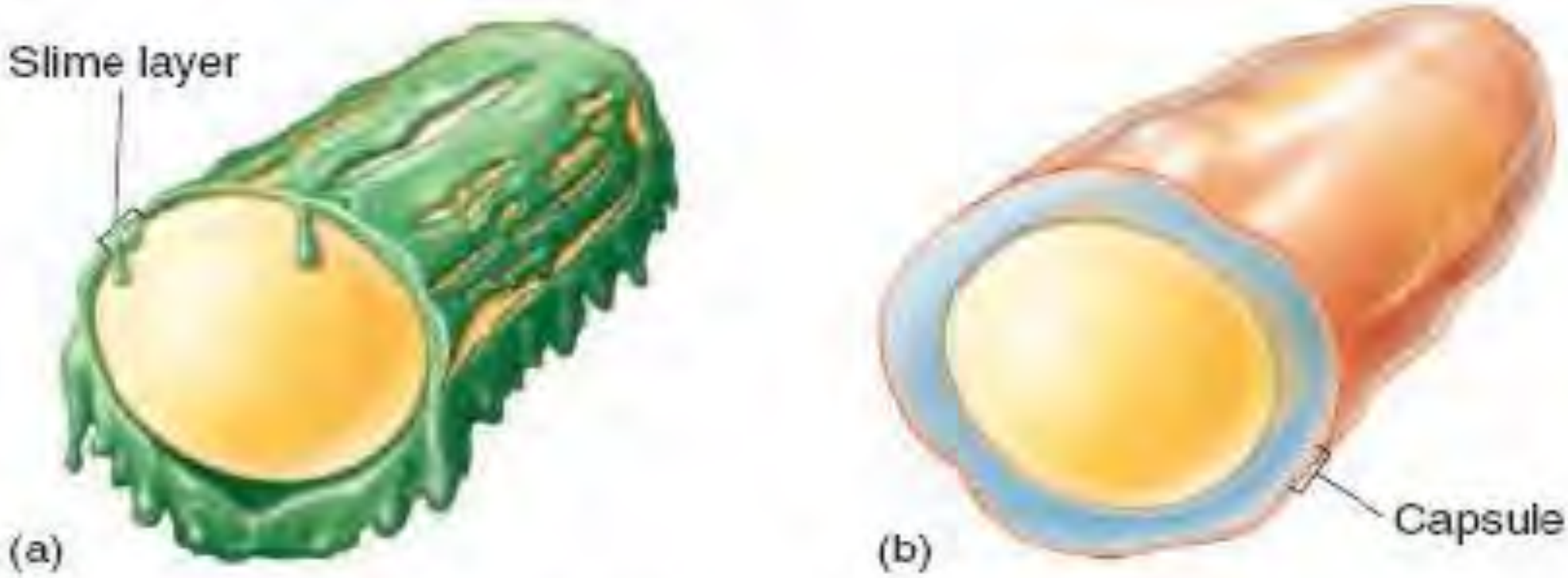
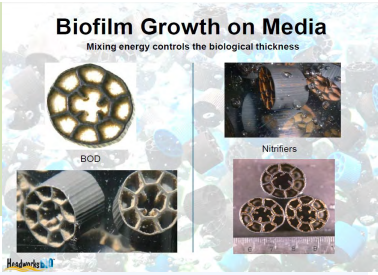
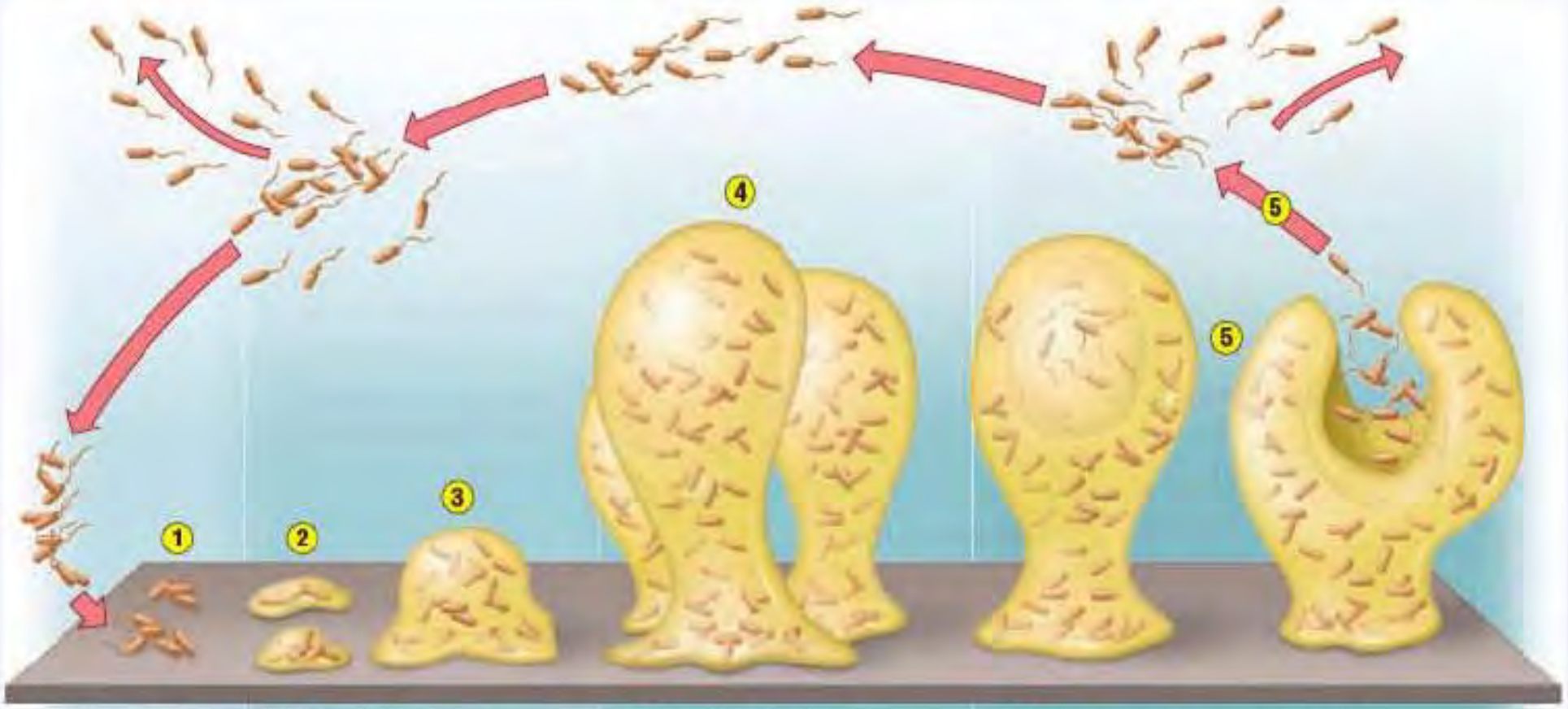


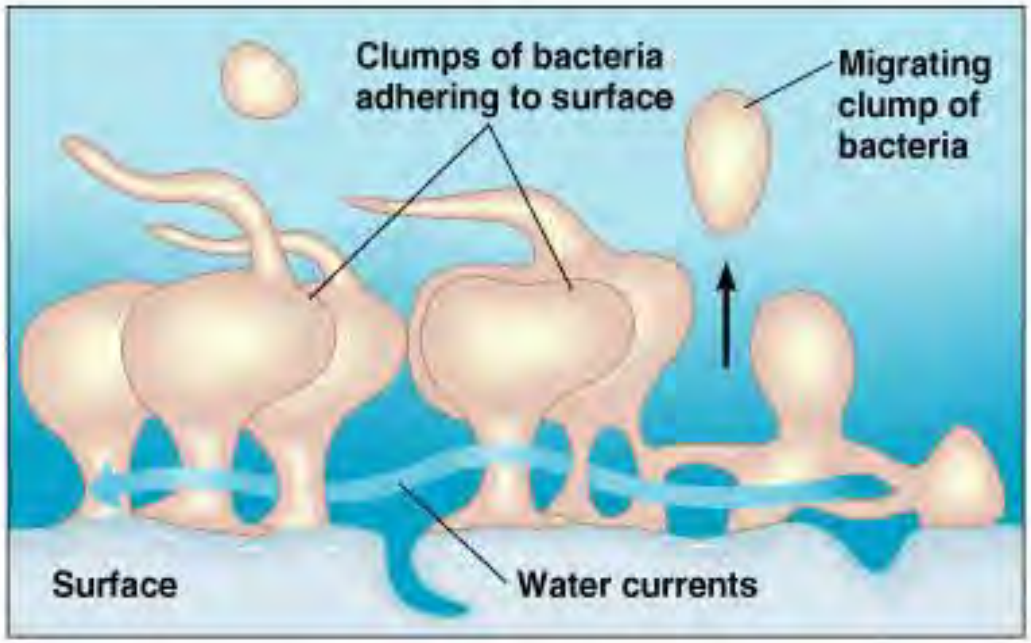
Figure 4.10 Types of glycocalyces seen through cutaway views of cells. (a) The slime layer is a loose structure that is easily washed off. (b) The capsule is a thick, structured layer that is not readily removed.

- **Biofilms**- are populations or communities of microorganisms
- Attach and grow on a solid surface that has been exposed to water.
- Mostly, they are encased in an extracellular polysaccharide that they synthesize





1. Attachment of free planktonic cells to surface
2–3. Growth of cells, synthesis of extracellular matrix, stable colonization of surface
4. Maturation of biofilm formation of thick layered deposits containing sessile cells
5. Mature biofilm completes cycle by dispersing planktonic cells, which swim free to start the process again in new habitats



(a) Water currents move, as shown by the blue arrow, among pillars of slime formed by the growth of bacteria attached to solid surfaces. This allows efficient access to nutrients and removal of bacterial waste products. Individual slime-forming bacteria or bacteria in clumps of slime detach and move to new locations.

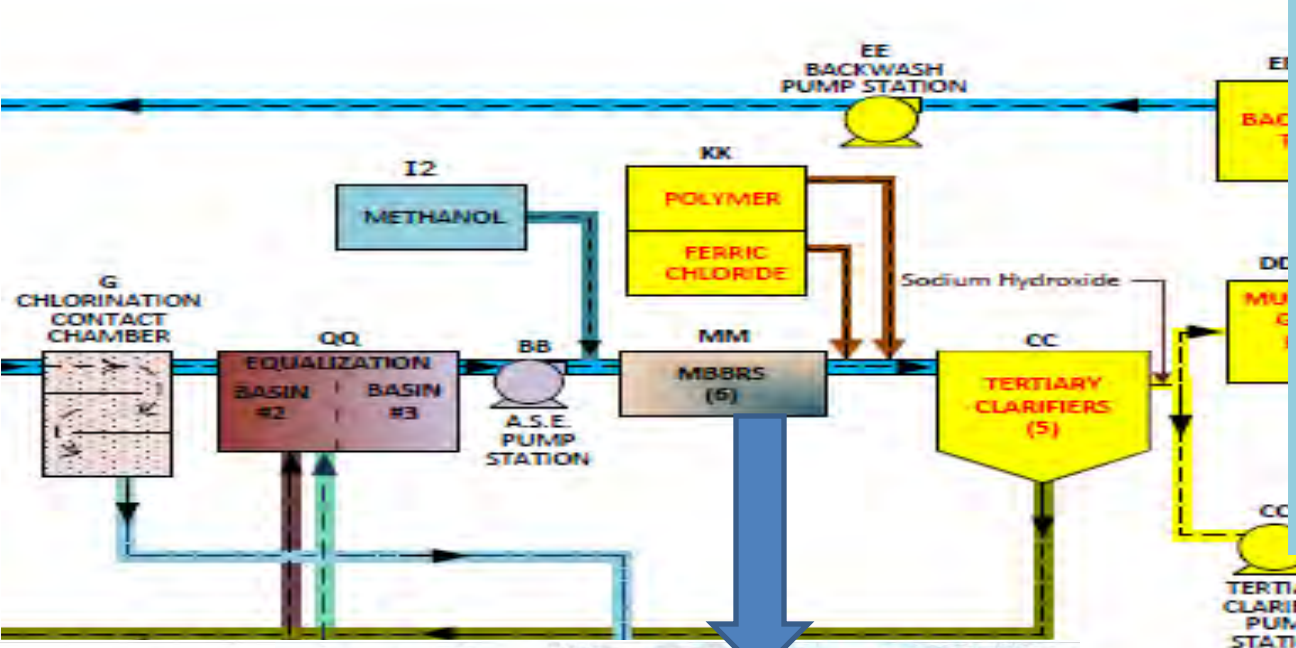
(b) A bacterial biofilm that formed on a steel surface over two months in an industrial water system. The large oval bodies are diatoms that became entrapped in the sticky biofilm.

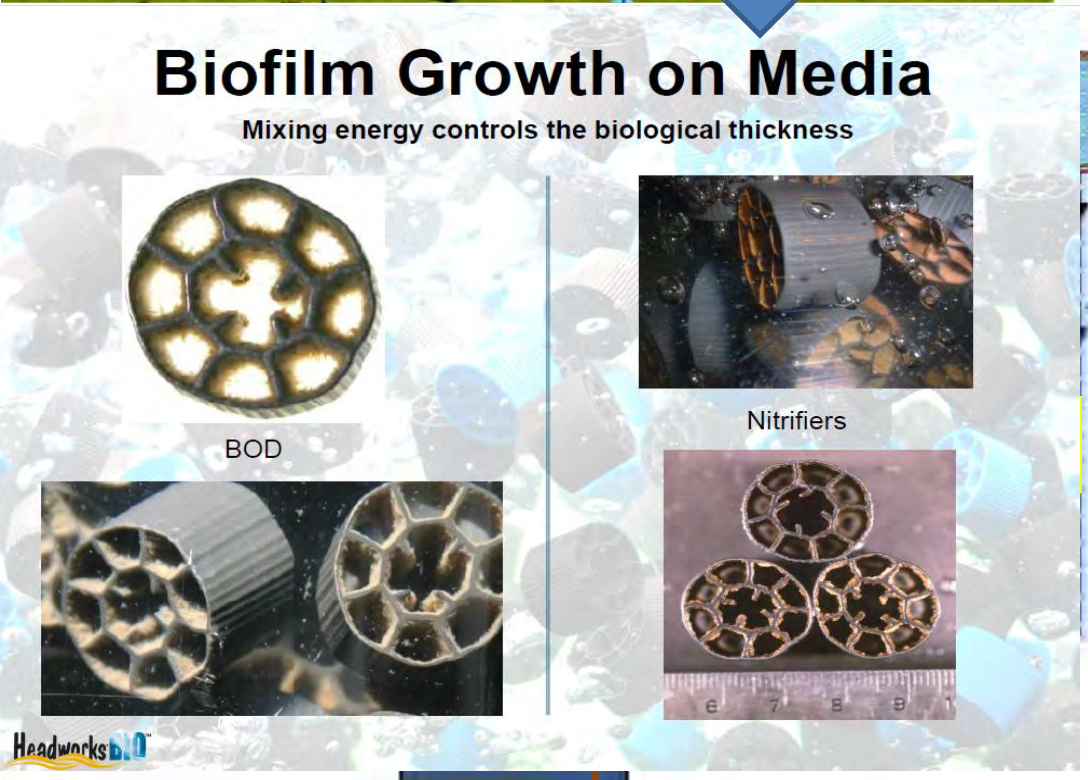
- Cell to cell communication or *quorum sensing*
- *A*llows bacteria to coordinate their activity and group together into communities
- This provide benefits not unlike those of multicellular organisms

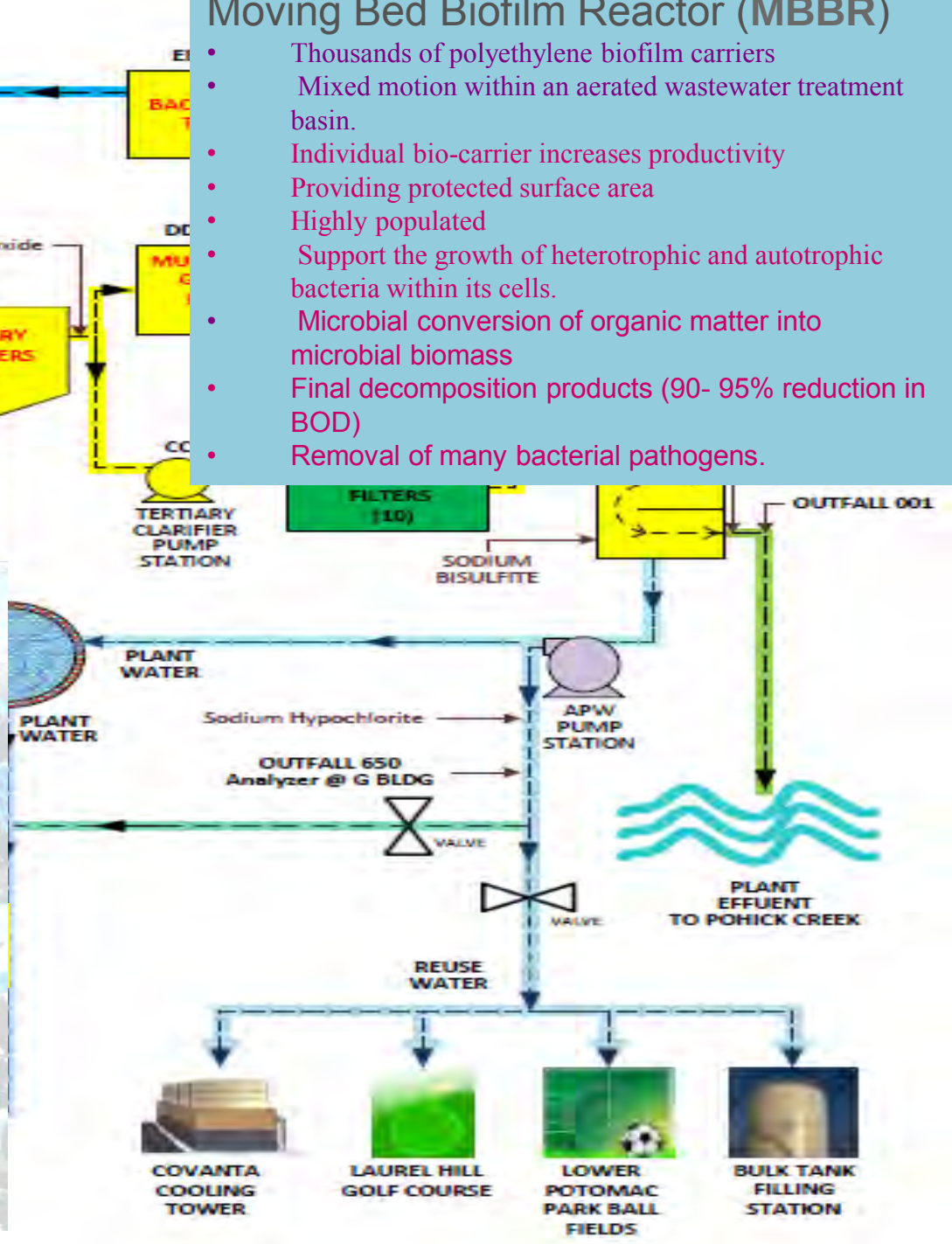
Figure 27.11

Moving Bed Biofilm Reactor (MBBR)

- Thousands of polyethylene biofilm carriers
- Mixed motion within an aerated wastewater treatment basin.
- Individual bio-carrier increases productivity
- Providing protected surface area
- Highly populated
- Support the growth of heterotrophic and autotrophic bacteria within its cells.
- Microbial conversion of organic matter into microbial biomass
- Final decomposition products (90- 95% reduction in BOD)
- Removal of many bacterial pathogens.



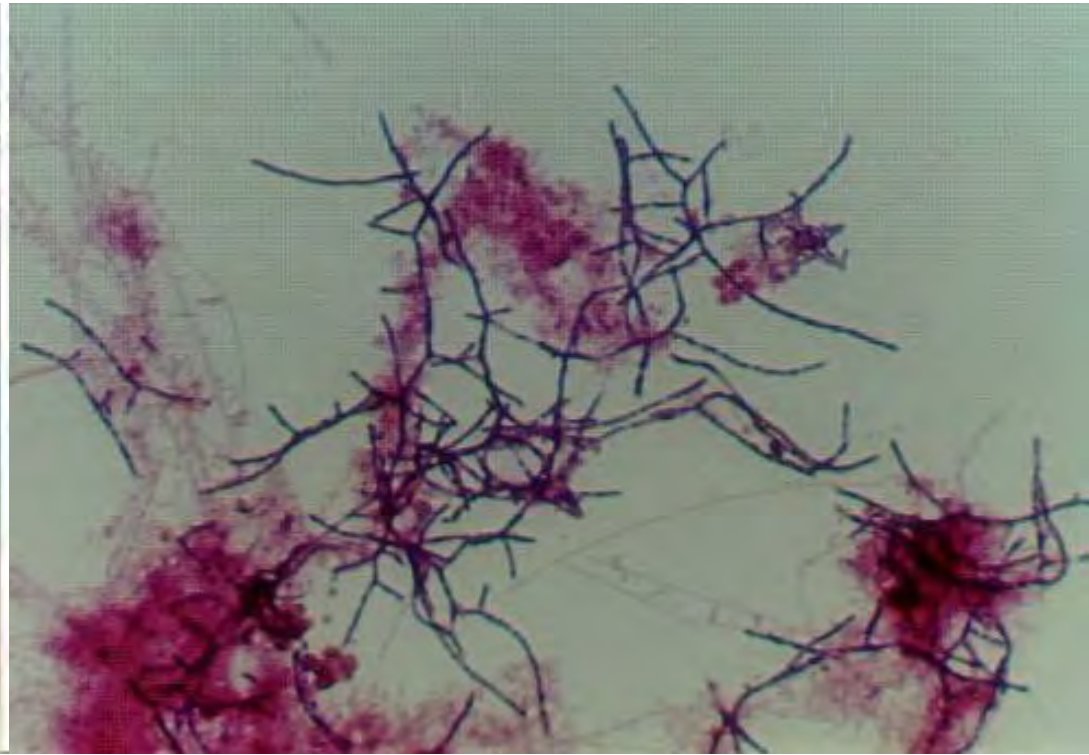


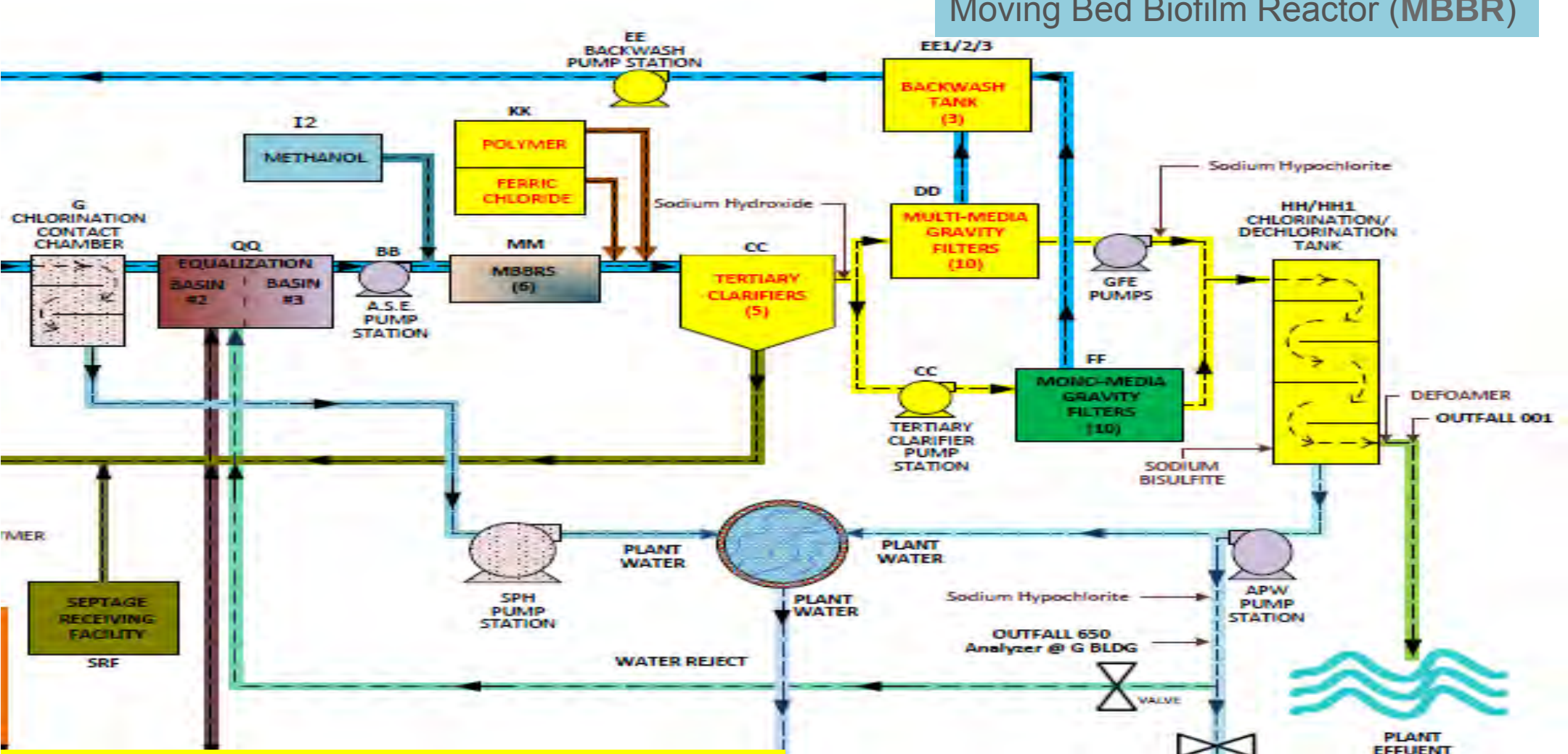


FOAMING IN OUR PLANT

Nocardia: http://www.lewwtp.org/educational-center/microbiology-photos

- **Filamentous bulking and foaming-** *Nocardia sp*.
- *Nocardia*: A filamentous organism that has a hydrophobic (water repellant) waxy nature.
- Nocardia floats under aeration making it difficult to waste from the system.
- The foam layer that results entraps other organisms.





Tertiary treatment: biological and chemical removal of inorganic nutrients (e.g., N and P) to reduce eutrophication of receiving ecosystem, virus removal or inactivation, trace chemical removal.

3. Microbiology- Water Quality Testing
INDICATOR ORGANISMS

- Historically- concern about water purity→ related to the transmission of disease
- Tests have been developed to determine safety of water
- The test for water purity in use today are aimed at detecting particular indicator organisms.

INDICATOR ORGANISMS

- An organism that can be readily cultured
- That indicates the presence of a pathogenic microorganism
- Or correlates to a health problem.

INDICATOR ORGANISMS

- Five criteria for an indicator organism:
- Consistently present in feces and at higher concentrations than pathogens.
- Should not multiply outside the human intestinal tract.
- Should be as resistant or more resistant than the pathogen to environmental conditions and to disinfection.
- Easy to assay (culture and quantify) and differentiate from other organisms.
- Environmental concentrations should correlate with pathogens or measurable health hazards.
-

INDICATOR ORGANISMS

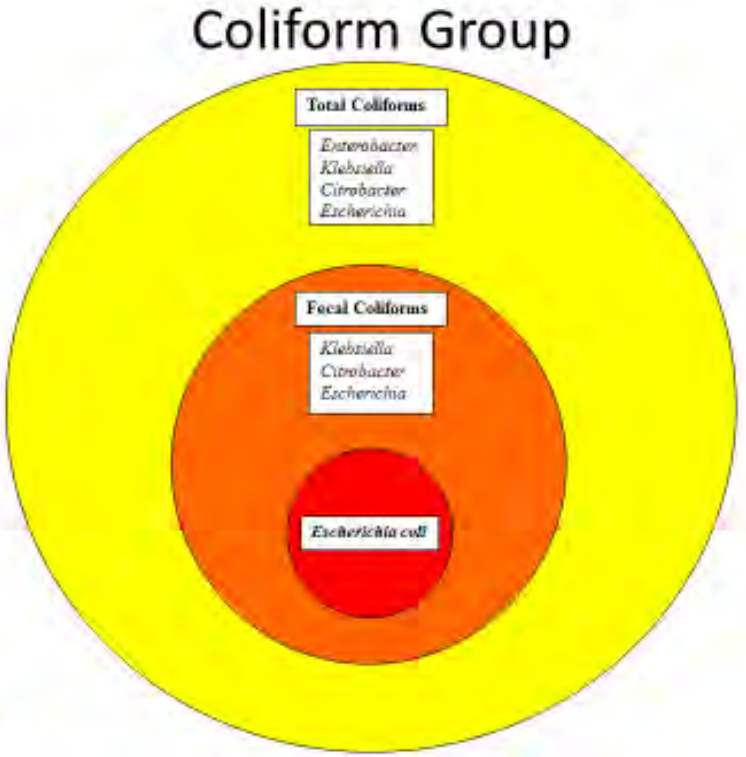
- In the United States- the usual indicator organisms are the coliform bacteria
- Defined as-
- Aerobic or facultative anaerobic
- Gram-negative
- Non–endospore forming,
- Rod-shaped bacteria that ferment lactose to form gas within 48 hours of being placed in lactose broth at 35°C.

INDICATOR ORGANISMS

- Some coliforms are not solely enteric bacteria
- They are most commonly found in plants and soil samples
- Many standards for food and water specify the identifications of fecal coliforms

TOTAL COLIFORMS

- EPA considers them a useful indicator of these pathogens.
- Health problems associated with these pathogens include diarrhea, cramps, nausea and vomiting
- The total coliform group is recognized in the drinking water standards of public health criteria.

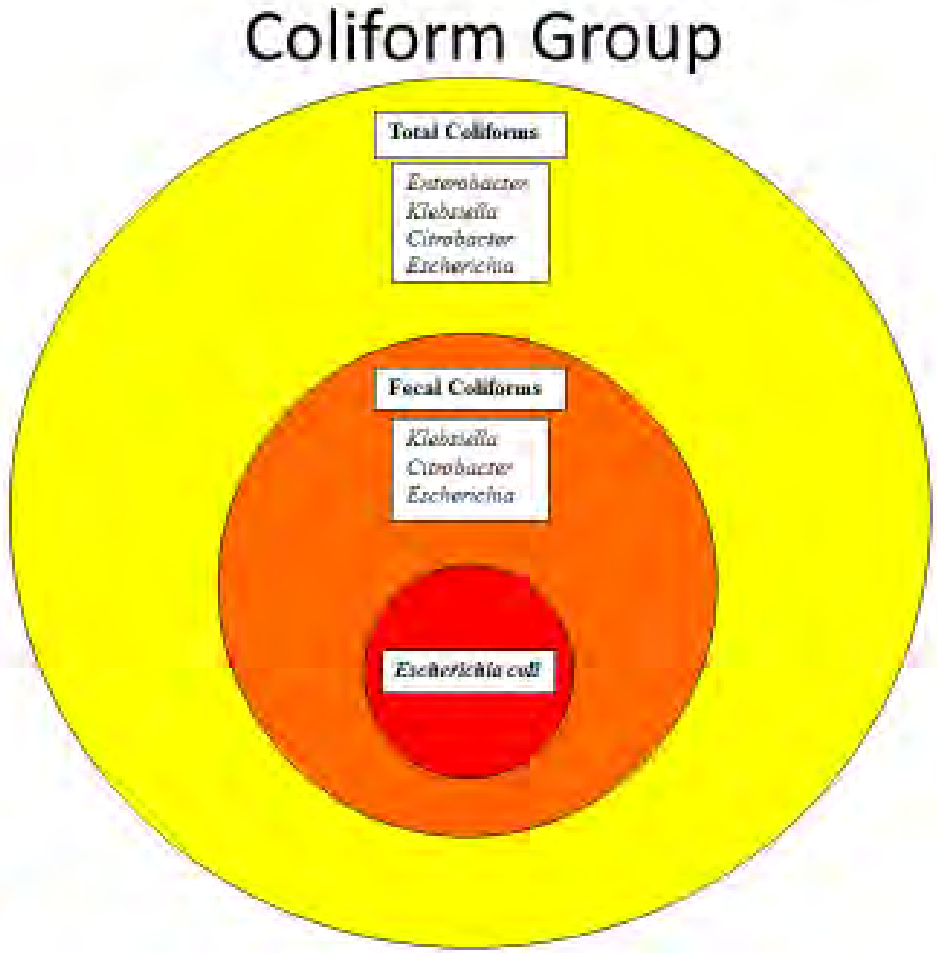


Fairfax Water

TOTAL COLIFORMS

- Total coliform counts give a general indication of the sanitary condition of a water supply.

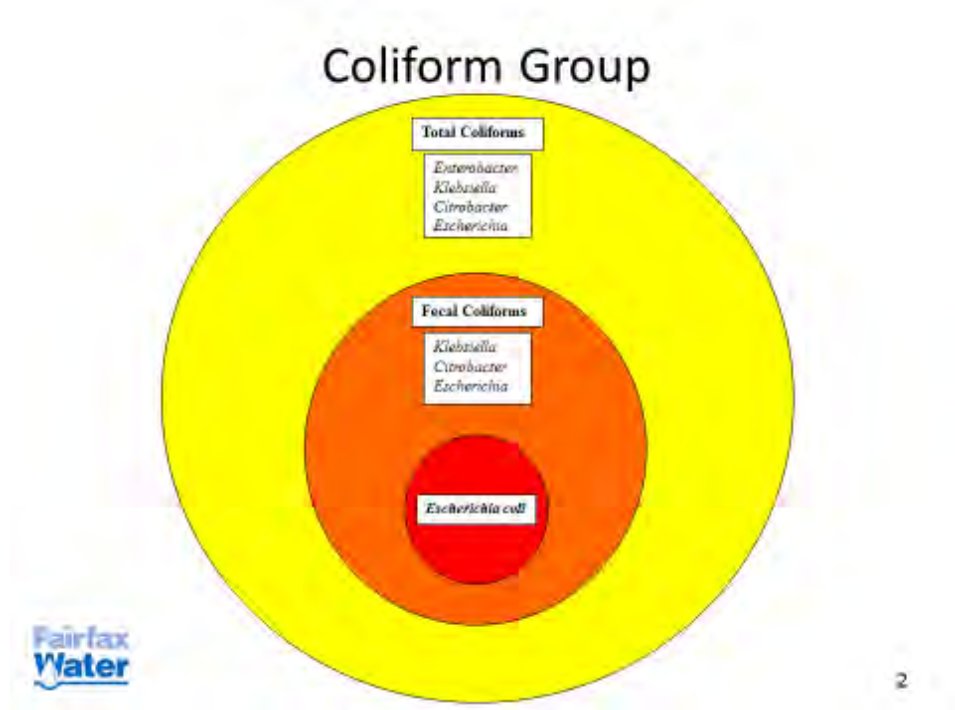
- http://www.health.ny.gov/environmental/water/drinking/coliform_bacteria.htm
- https://www.idexx.com/water/products/colilert.html



Fairfax Water

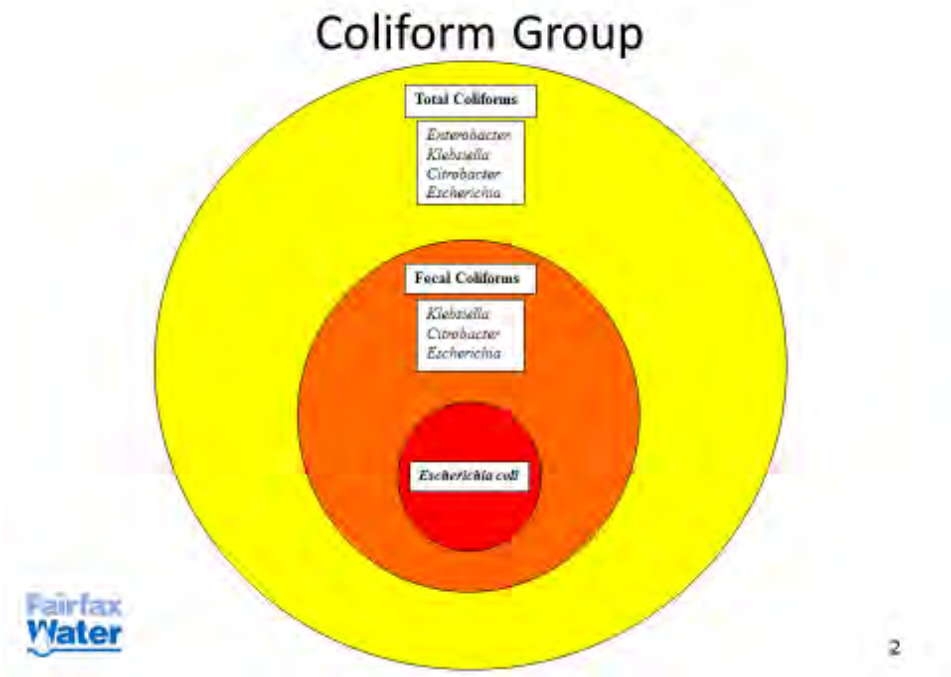
FECAL COLIFORMS

- Fecal coliforms are the group of the total coliforms
- Considered to be present specifically in the gut and feces of warm-blooded animals.



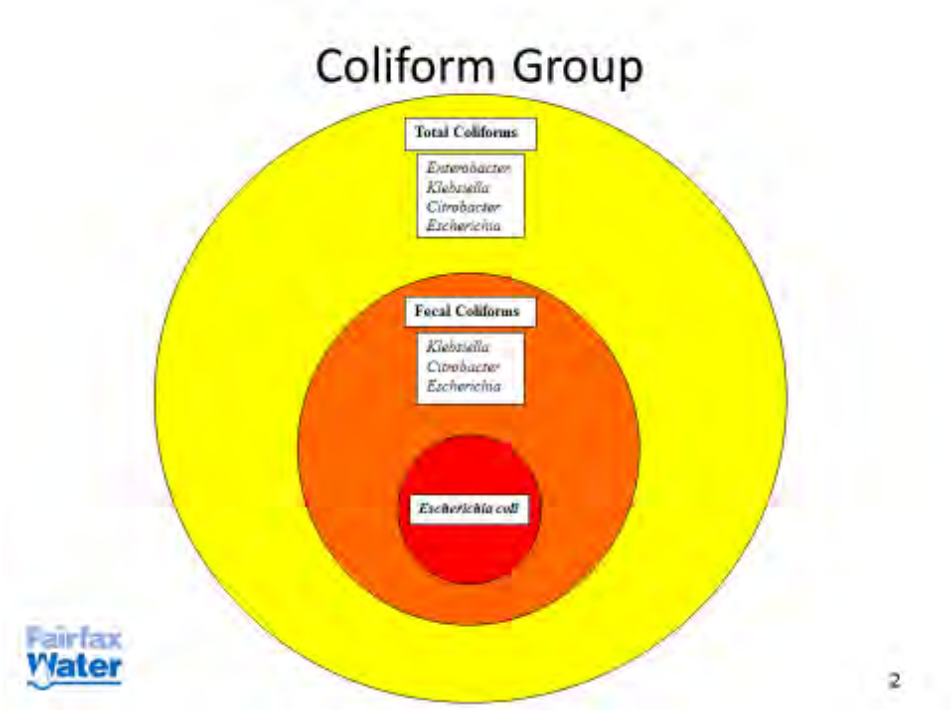
FECAL COLIFORMS

- Origins of fecal coliforms **are more specific** than the origins of the more general total coliform group of bacteria
- Fecal coliforms are considered a more accurate indication of animal or human waste than the



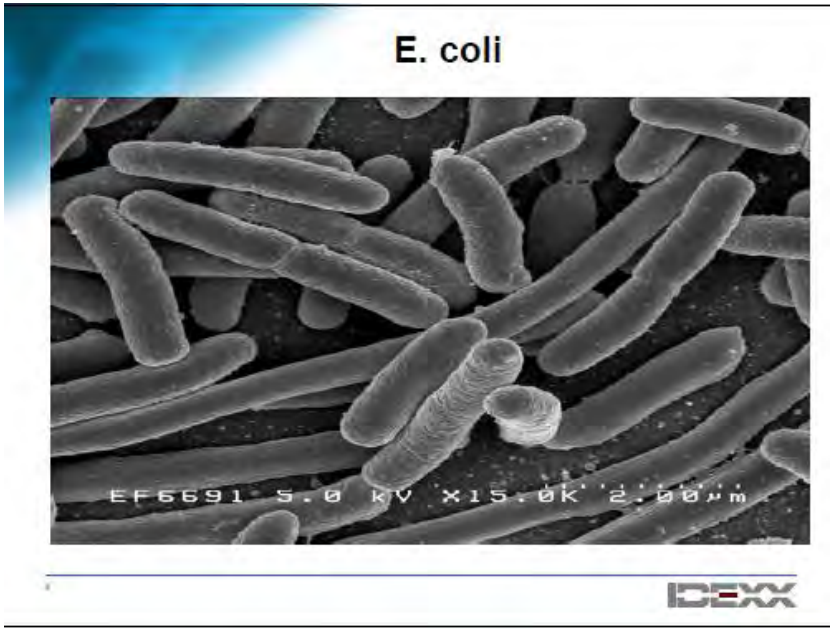
E. *COLI*

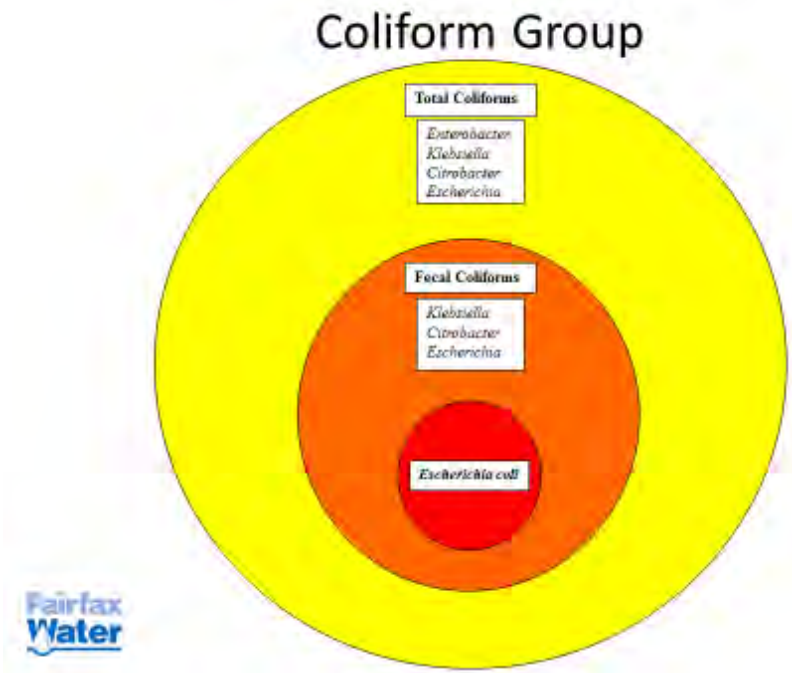
- Escherichia *coli* (E. *coli*) is the major species in the fecal coliform group.
- Only E. *coli* is generally not found growing and reproducing in the environment.



E. *COLI*

- E. coli is considered to be the species of coliform bacteria that is the best indicator of fecal pollution and the possible presence of pathogens.





COLIFORMS

- The method determining the presence of coliforms are based on the lactose-fermenting ability of coliform bacteria.
- The multiple-tube method- can be used to estimate coliform numbers by the most probable number (MPN) method
- The membrane filtration method- more direct method of determining the presence and numbers of coliforms.

COLIFORMS

- A newer and more convenient method of detecting coliforms, specifically the fecal coliform E. coli
- The use of media containing two substrates

In biochemistry, the substrate is a molecule upon which an enzyme acts

COLIFORMS

- Use of media containing the **two substrates**
- *o*-nitrophenyl-*β*-*D*-galactopyranoside (ONPG) and
- 4-methylumbelliferyl-*β*- *D*-glucuronide (MUG).

COLIFORMS

- Coliforms produce the enzyme *β*-galactosidase
- It acts on ONPG substrate and forms a yellow color indicating their presence in the sample.
- *ONPG- o-nitrophenyl-β-D-galactopyranoside*

E. *COLI*

- E. *coli is* unique among coliforms
- Almost always producing the enzyme *β*-glucuronidase
- Acts on MUG (4-methylumbelliferyl-*β*- *D*-glucuronide) to form fluorescent compound
- Glows blue when illuminated by long-wave ultraviolet light (Figure 27.16).

IDEXX VIDEO

TEST

- These simple tests, or variants of them, can detect the presence or absence of coliforms or E. *coli*
- Can be combined with the multiple-tube method to enumerate them.
- It can also be applied to solid media, such as in the membrane filtration method.
- The colonies fluoresce under UV light.

Microbes are essential to wastewater treatment technology

- Wastewater is teaming with microbes.
- Many of which are necessary for the degradation and stabilization of organic matter and are beneficial.
- On the other hand, wastewater may also contain pathogenic or potentially pathogenic microorganisms, which pose a threat to public health.

Microbes are essential to wastewater treatment technology

- Waterborne and water-related diseases caused by pathogenic microbes→ among the most serious threats to public health today.
- Waterborne diseases whose pathogens are spread by the fecal-oral route (with water as the intermediate medium) can be caused by bacteria, viruses, and parasites (including protozoa, worms and rotifers).

TEST

- Water quality has inspired development of tests designed to measure its suitability for drinking, bathing, and release back to the environment
- There are so many potential pathogens that it is impractical to test for them all.
- Because of this, tests have been developed for indicator organisms.
- These are organisms that are present in feces (or sewage), survive as long as pathogenic organisms, and are easy to test for at relatively low cost.

Benefits Summary- microorganism

- Microorganism have diverse capabilities to degrade organic material- are exploited for the treatment of wastewater (sewage).
- Secondary wastewater treatment relies on the power of microorganisms to breakdown solid waste into smaller molecules that other microbes can convert into nutrients.

Benefits Summary- microorganism

- Nutrients are further converted either by nitrifying bacteria into nitrate and then denitrifiers convert the nitrate to N2 gas.
- Small organic acids are eventually converted into methane gas by methanogenic archaea.

Benefits Summary- microorganism

- The effluent released into the environment should have a low BOD and low numbers of pathogens.
- Monitoring is done to quantify indicators of pathogens and BOD to make sure the environment is safe for humans and wildlife.

Fairfax County Industrial Waste Section

Noman M. Cole, Jr. Pollution Control Plant

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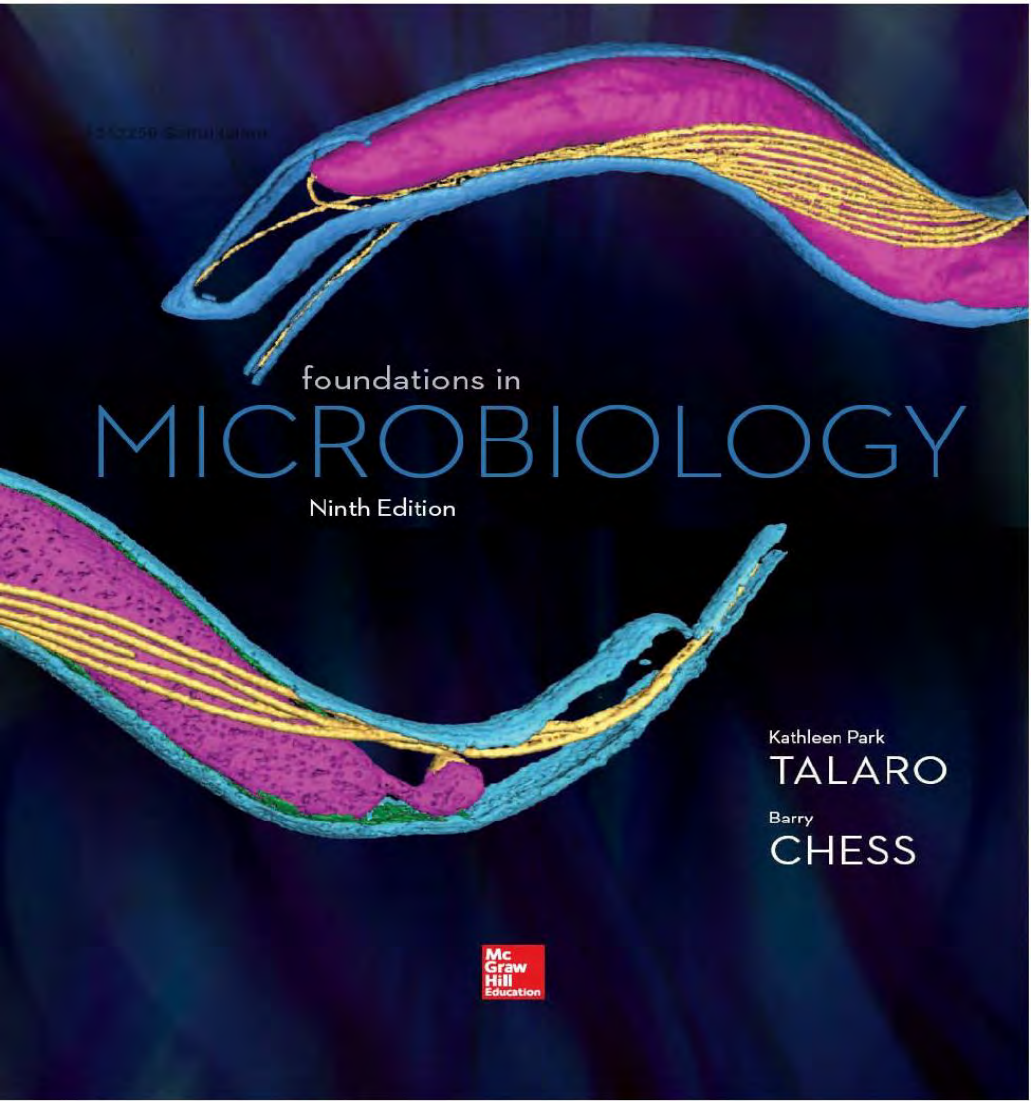
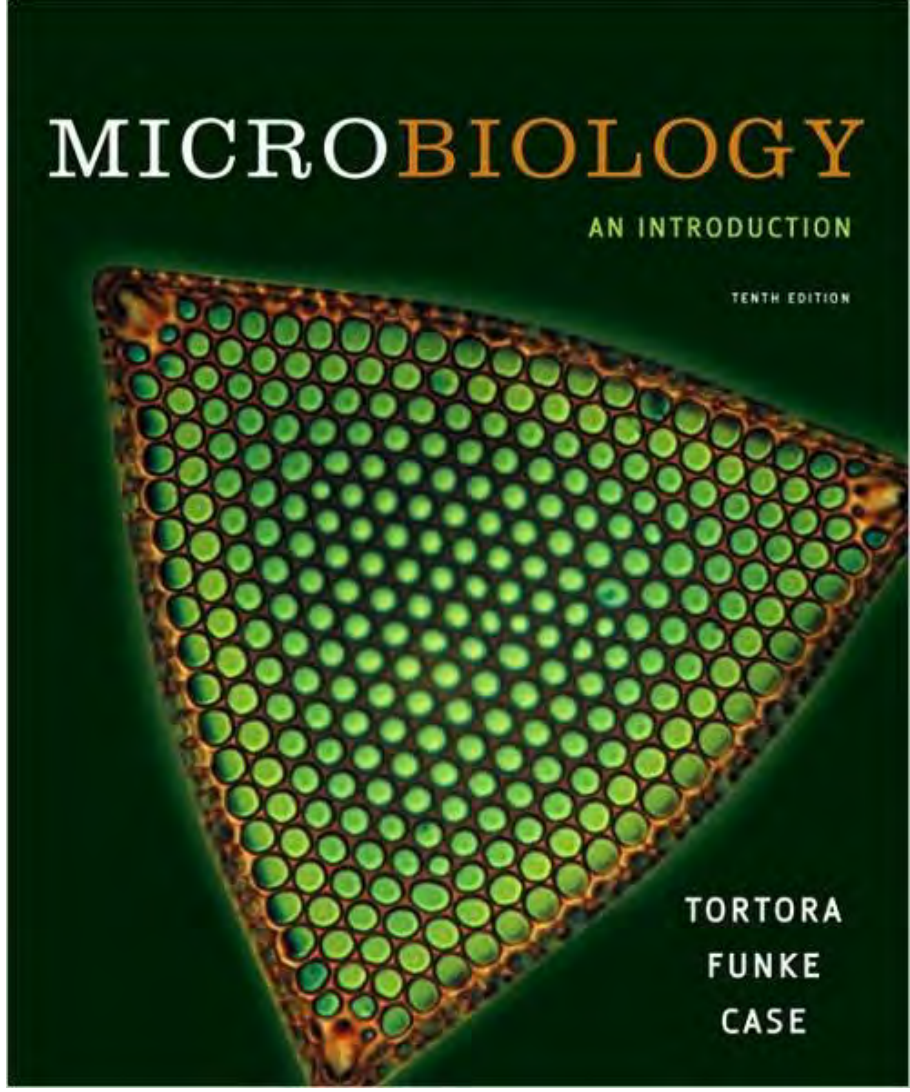
Lorton, Virginia 22079

703-550-9740 ext. 418

Check out our exciting web page:

www.fairfaxcounty.gov/dpwes/wastewater/industrialwaste/





The information, diagrams, flow chart in this presentation are taken from different textbooks, various websites, Noman M. Cole Pollution Control Plant permit and other online media- Disclaimer

questions????

Next Speaker is John Allen