Clean Water Professionals of Kentucky & Tennessee

An Introduction to an Unconventional Microbiological Monitoring Tool.

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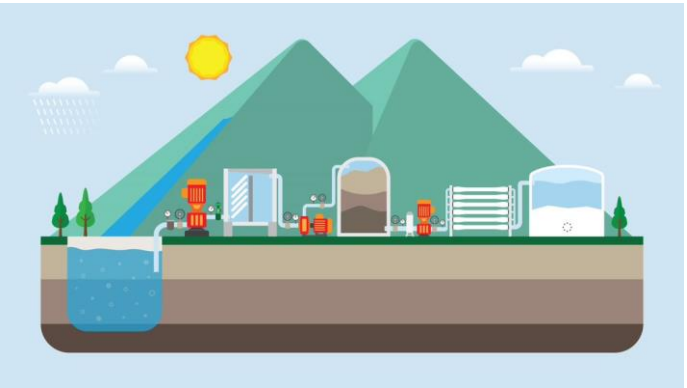


LUMINULTRA® microbial monitoring

HACH

The Water Sector

Drinking Water

Run a process in which pollutants are removed from water making it safe to drink.

Water leaving the facility is of high quality however issues can arise within the distribution system:

- Loss of residual
- Biofilm Formation
- Nitrification

Water Resource Recovery

Removing pollutants/nutrients from water making it safe to return to the environment.

Microorganisms are used to remove different nutrients. However, can be affected by things such as:

- Low Amount of Living Organisms
- Toxicity
- Bulking

The Water Sector – The Problem

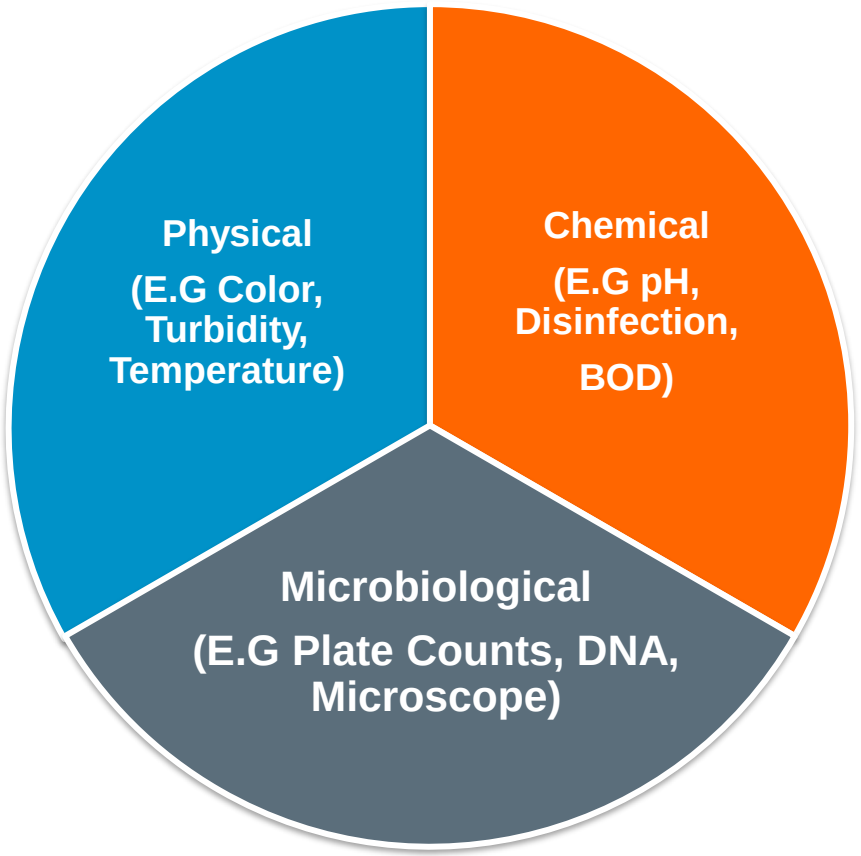
Drinking Water

- Loss of Residual
  - What is the disinfection going after?
- Biofilm Formation
  - What can occur if the disinfection residual drops?
- Mitigation Effectiveness
  - How did the mitigation action effect the system?

Water Resource Recovery

- Low Amount of Living Organisms
  - How does the quantity of living organisms compare to the total solid?
- Toxicity
  - Has something entered the system causing the organisms to react badly?
- Bulking
  - Is something promoting filamentous organism growth?

The Water Quality Toolbox



The water quality toolbox within the drinking water industry is made up of 3 parts:

- Physical Analysis
- Chemical Analysis
- Biological Analysis

Each section brings unique and valuable insight to how the system is performing

The Water Sector – The Tools

Drinking Water

- Physical
  - Turbidity
  - Color
  - Alkalinity
- Chemical
  - Disinfection Residual
  - pH
  - Hardness
- Microbiological
  - Plate Counts☆

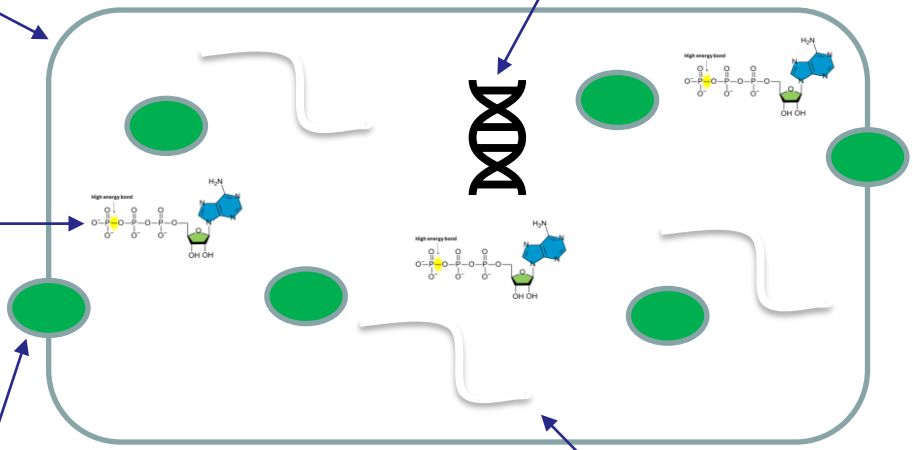
Water Resource Recovery

- Physical
  - Settleability
  - Total Suspended Solids
  - Temperature
- Chemical
  - BOD☆
  - Nitrogen
  - Phosphorous
- Microbiological
  - Microscope
  - DNA☆
  - Plate Counts☆

Inside the Prokaryote Cell

DNA
Highly Specific and Readily Targetable

Membrane (Lipid)
Typically Not Specific



ATP Molecule
Energy Carrier Non-Specific

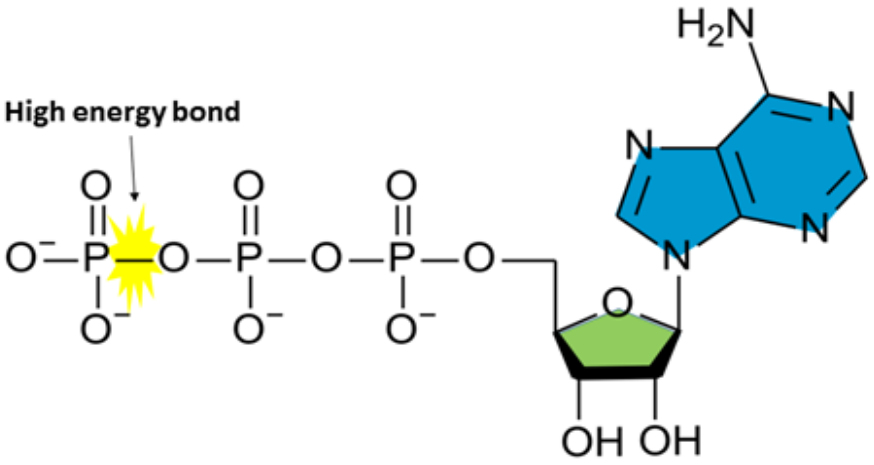
Protein/Enzyme
Can be Specific But Need Antibodies or Other Recognition Molecules

RNA
Specific but Difficult to Handle

What is ATP?

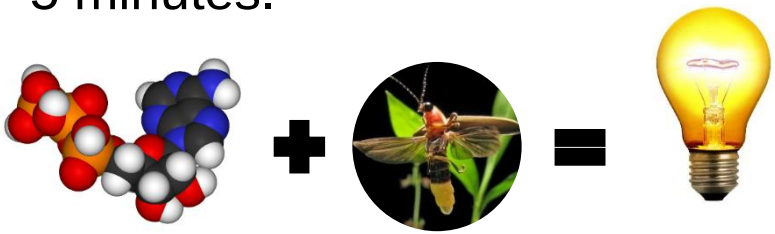
ATP = **A**denosine **Tri**p**h**osphate

- Primary energy carrier of all living cells.
- Energy is stored in the third Phosphate bond.
- If the cells needs energy it will cleave that bond forming ADP.



How is ATP Measured?

- As seen in the tails of fireflies, the reaction of ATP with Luciferase emits light.
- This light is measured using a luminometer and reported in RLU's.
- The extracting and analysis of ATP takes ~ 5 minutes.



ATP **Luciferase Enzyme (Luminase™)** **Light**

$$ATP + O_2 + luciferin \xrightarrow{Mg^{++}_{Luciferase}} AMP + PP_i + oxyluciferin + light$$

ATP in the Water Sector

Drinking Water

Why would I use a non-specific microbial test?

- Identify areas of total biological risk.
- Assess if turbidity is physical or biological.
- Troubleshoot and see how mitigation action affects the system.

Water Resource Recovery

Why would I use a non-specific microbial test?

- Identify the % of TSS that is living.
- See if the total amount of organisms suddenly decreases.
- Determine the percentage of organisms that are filamentous.

Industry Standard – HPC

- HPCs take at least 48 hours to obtain results.
- **HPCs are known to detect <1% of the total population[1].**
- Information is only provided on organisms that can grow…
  - …in the media used
  - …at the temperature provided
  - …within the incubation time allowed
- Injured or unhealthy cells grow more slowly or not at all.

[1] Maki et al., 1986; Siebel et al., 2008

Drinking Water – Finding the Source

A Customer Called:

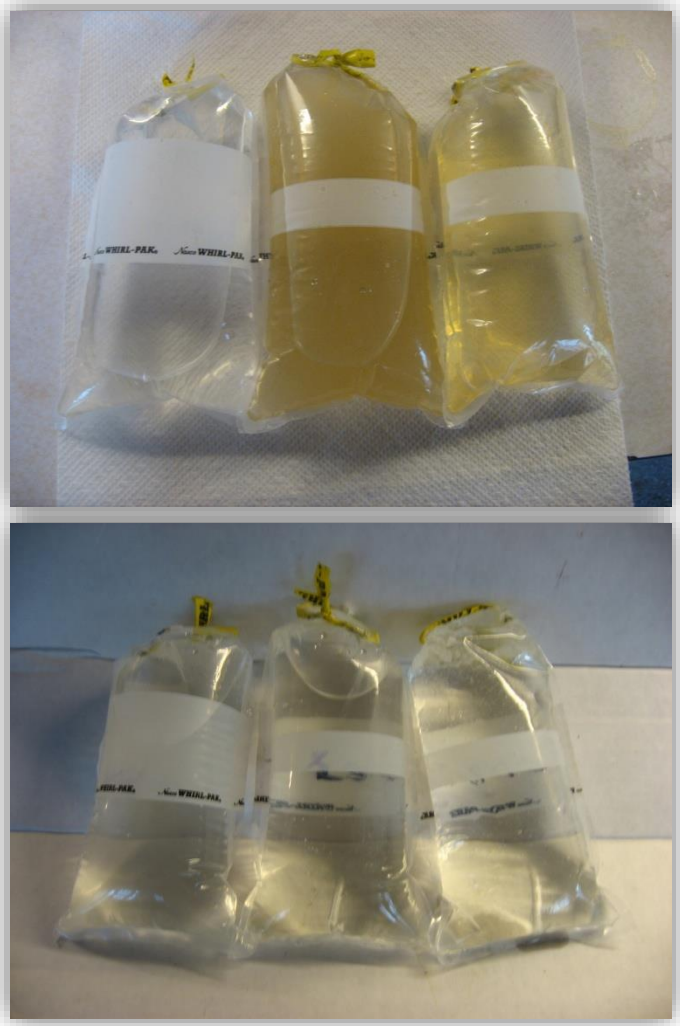
- Water quality leaving the facility was excellent!
- Disinfection residuals quickly disappeared without a clear explanation.
- ATP was used to troubleshoot.

Resolution:

- An un-listed valve was leaking allowing biofilm to build up and enter the system.

Drinking Water – Turbidity

- Visual inspection and turbidity can indicate high risk in some case.
- However in other cases, immediate assessment is not so easy.
- For samples such as this, one cannot quickly assess risk and must therefore wait until microbiological tests are done.



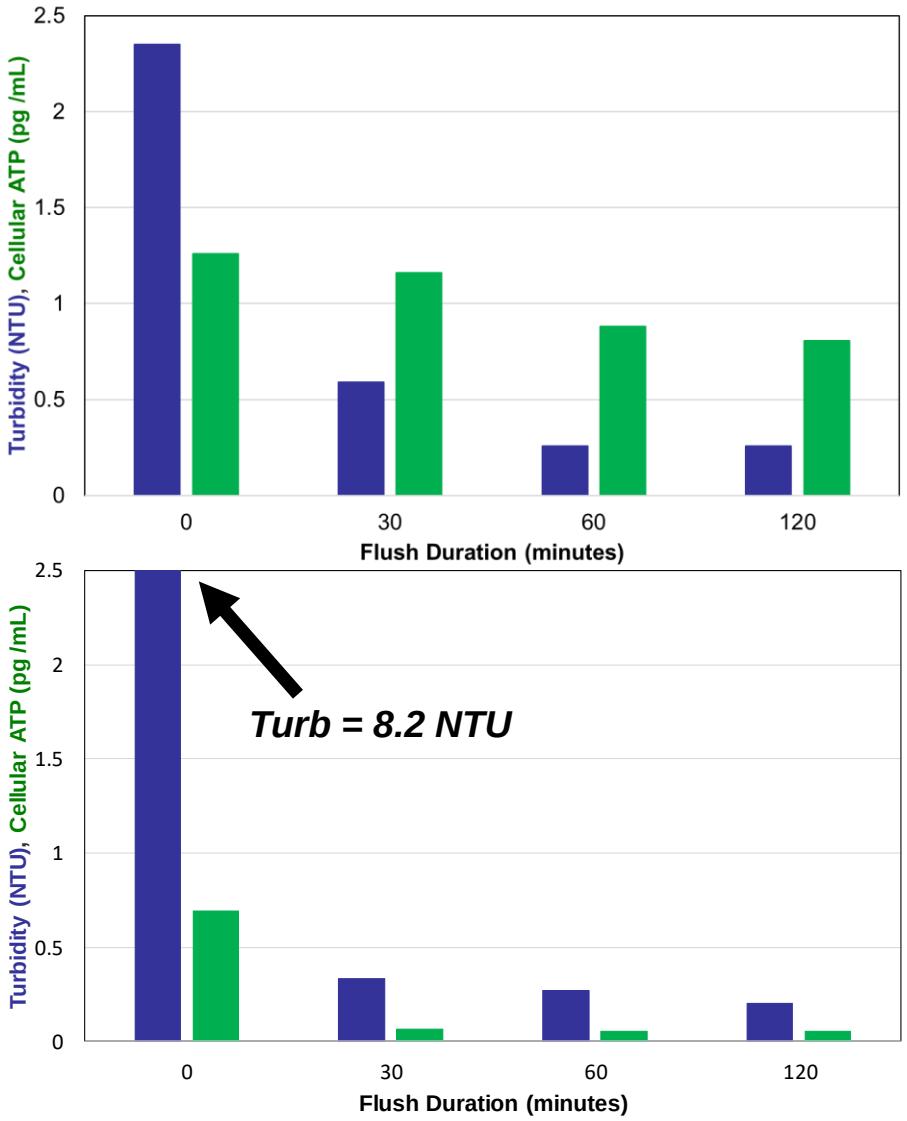
Drinking Water – Turbidity

Scenario 1:

- The turbidity + biological activity is high pointing to signs of biological issue.
- After flushing for 30 minutes, the turbidity dropped while the ATP remained high.

Scenario 2:

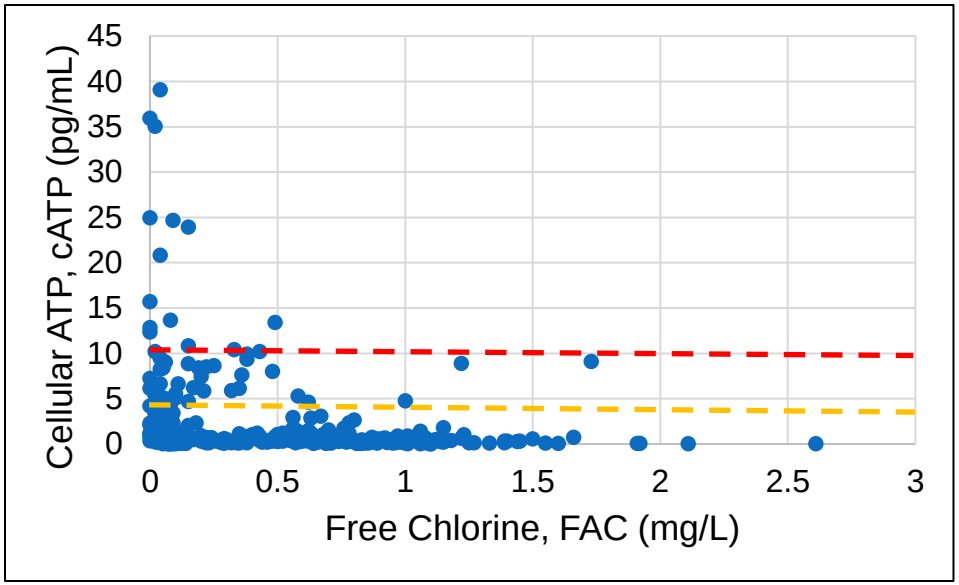
- Turbidity was at 8.2 while the ATP level was quite low indicating this is not a biological issue.
- After flushing 30 minutes, both measurements are low.



Drinking Water – Disinfection

Total Chlorine:

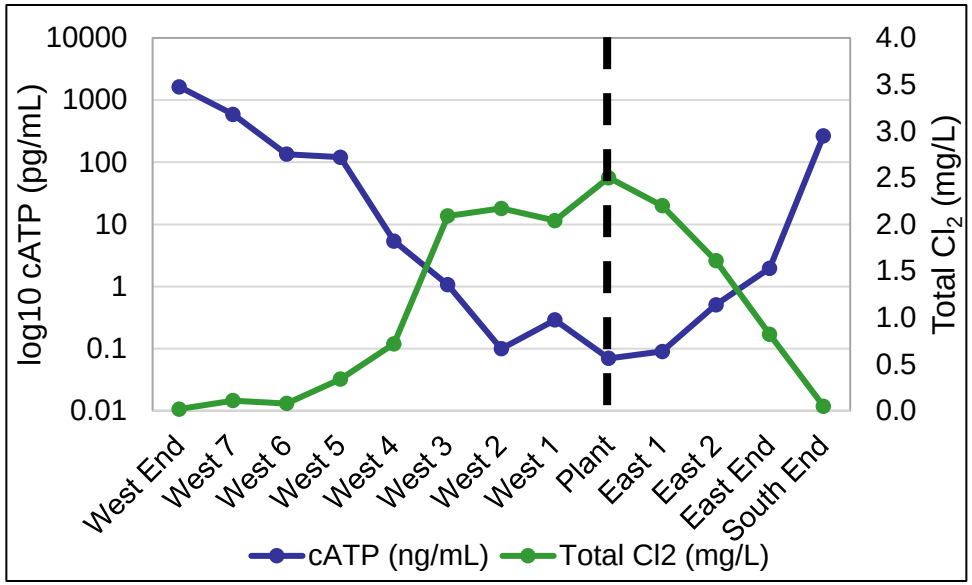
- As expected, there is a strong relationship between free chlorine and biological activity.
- However, Free Chlorine does not always indicate low biological activity.
- Microorganism can grow and thrive within biofilms that form an EPS layer protecting it from disinfection.
- EPS = Extracellular Polymeric Substances



Drinking Water – Disinfection

Distribution System Audit:

- Both chlorine and ATP results were taken at points moving away from the plant.
- Shown in West 3, while Chlorine is still high the biological activity has already raised to a high-risk level.
- It was determined that actions needed to be taken in the west end.



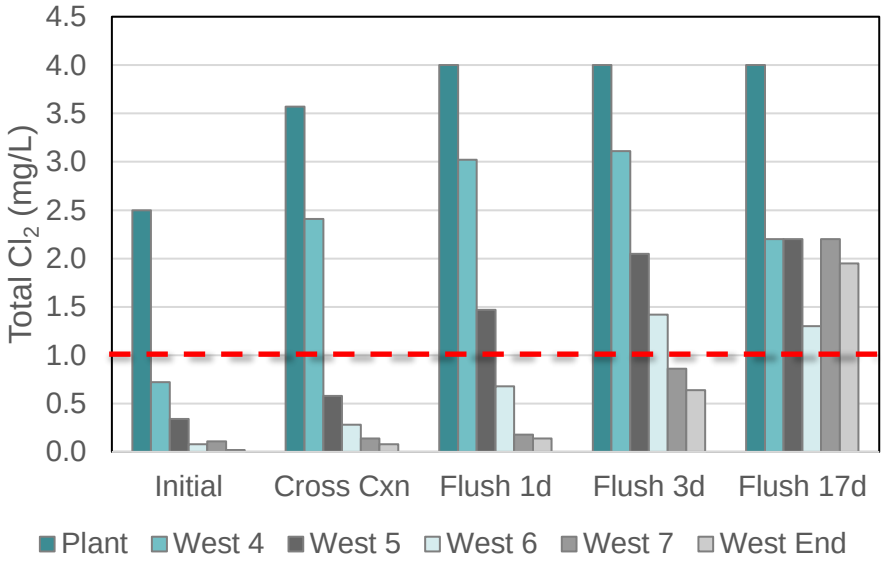
Drinking Water – Disinfection

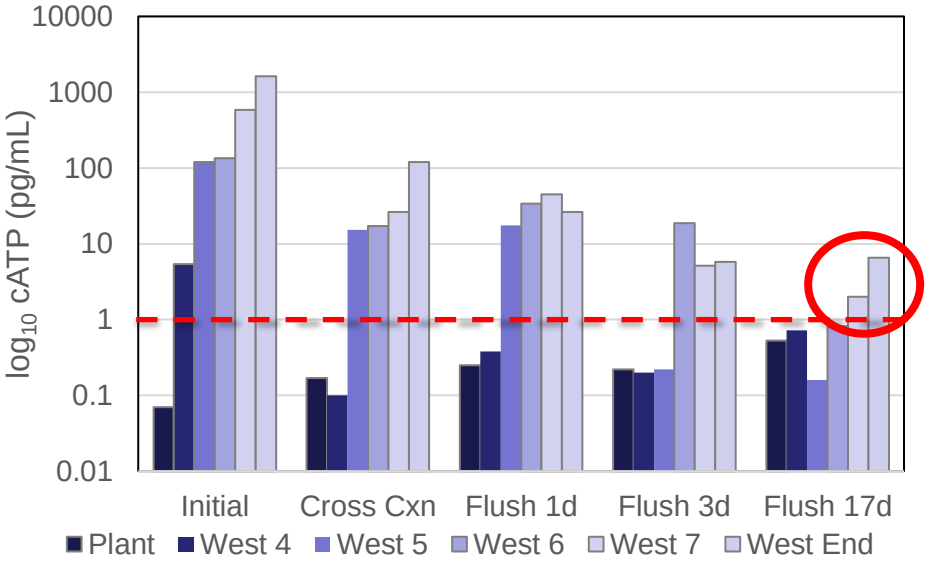
Chlorine:

- As the system is flushed, the chlorine increases across the west end.
- With a goal of 1.0 mg/L achieved, the flushing could stop.

ATP:

- Biological activity decreased during the flush as expected.
- However, while chlorine indicated the flushing could stop, ATP told a different story.





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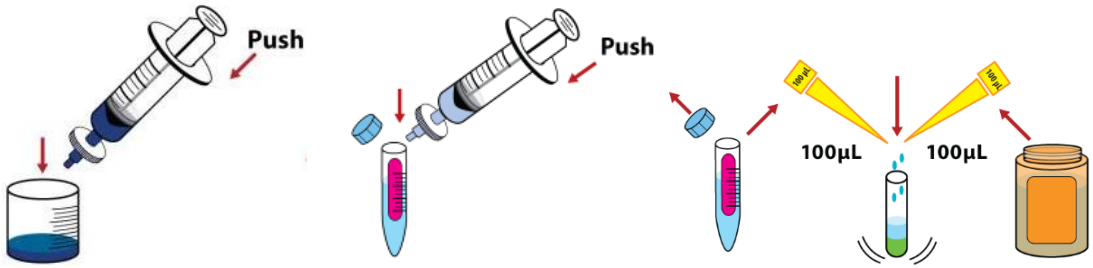
How to Take a Test – Drinking Water

- Filter the water to catch the organisms.
- Push an extraction fluid through the filter and into a dilution tube.
- Mix the dilution fluid with the firefly enzyme and place into a luminometer to take a reading.
- Mix the firefly enzyme with the calibration fluid to measure the enzyme strength and convert the units to a concentration.



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Water Resource Recovery

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- Identify the % of TSS that is living.
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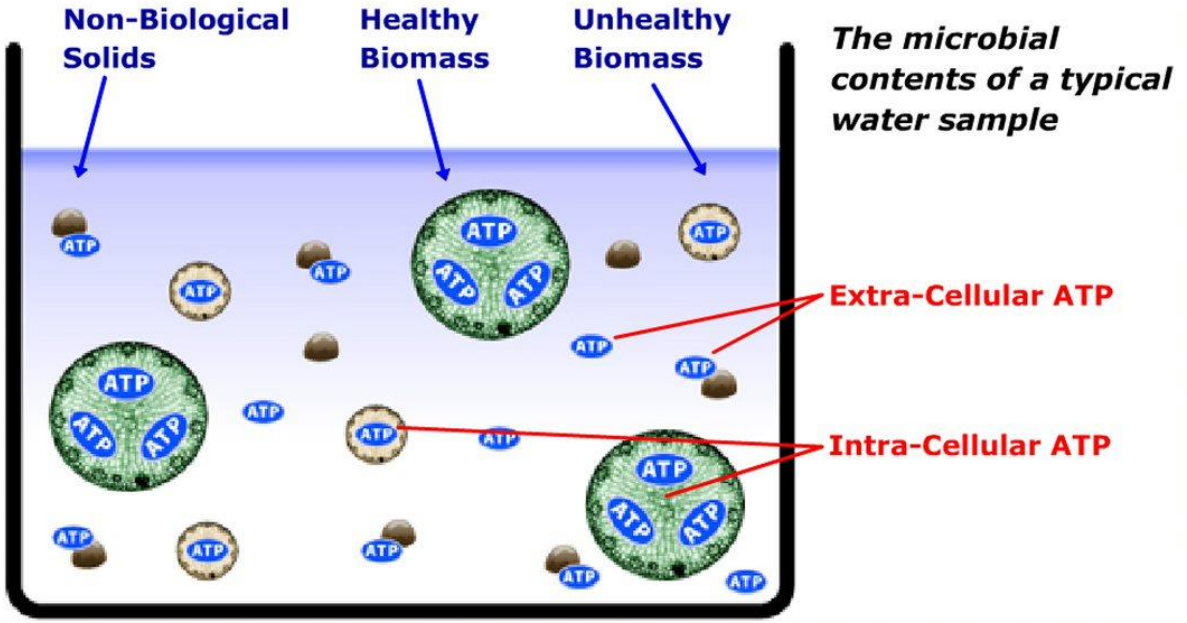
Water Resource Recovery Sample

Along with organics and inorganics, there are a few different types of ATP molecules that need to be identified:

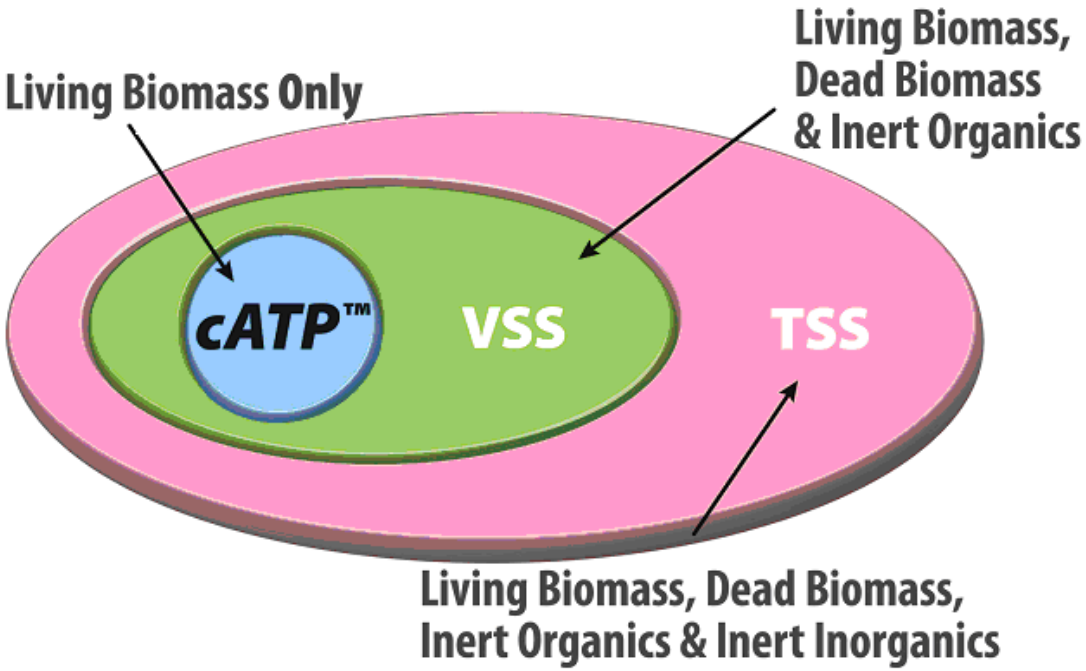
- Cellular ATP (cATP): ATP found within living organisms.
- Dissolved ATP (dATP): ATP found in the environment.
- Total ATP (tATP): The combination of cellular and dissolved ATP.



Water Resource Recovery – TSS/VSS

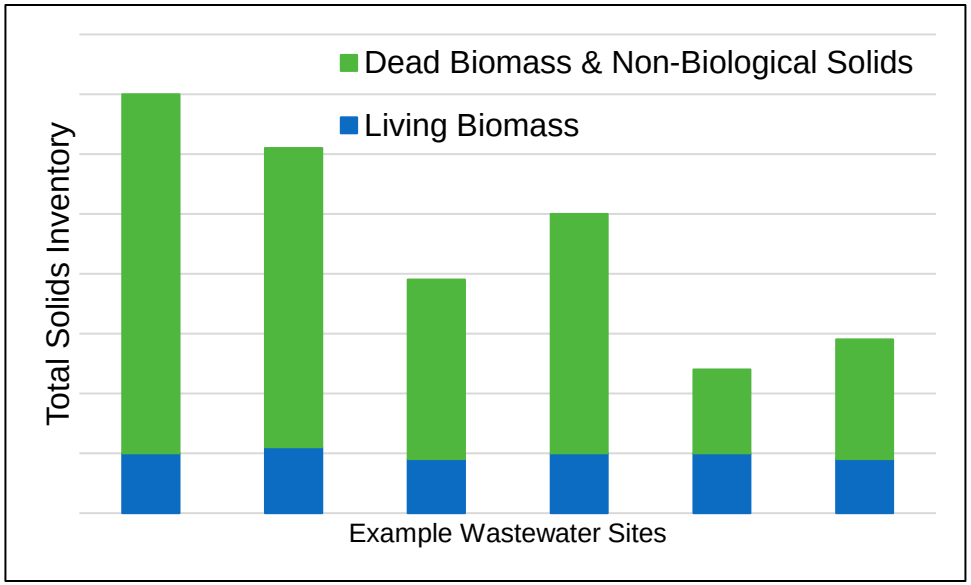
While these parameters are useful for identifying the flow of solids, they have some inherent interferences when looking to measure just the biological population.

Due to these interferences, they will react slower to change.



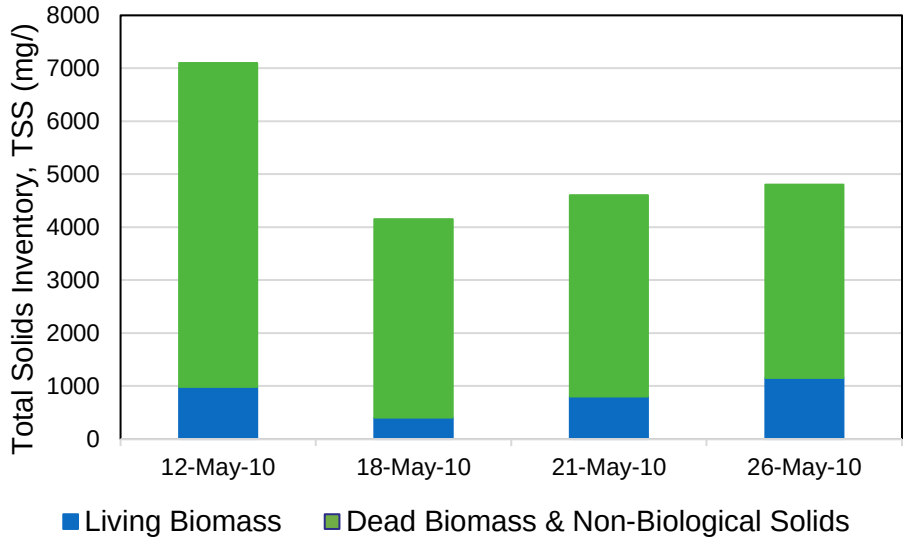
Total Solids Inventory

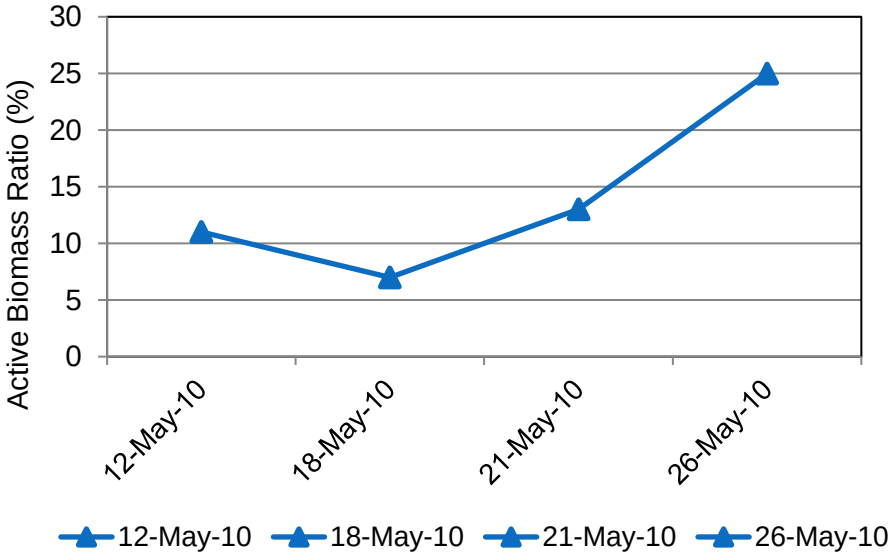
- What are some of the benefits of having a higher percentage of active biomass?
  - Better Setting
  - Reduced Bulking
  - Improved O2 Transfer Efficiency



Improving the Active Biomass Ratio

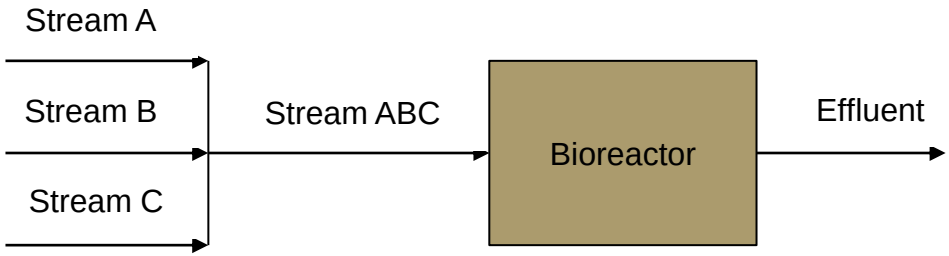
- A plant was experiencing issues with bulking.
- The typical solids inventory was ~ 7000 mg/L
- Through some scheduled and closely monitored wasting events the:
  - Total solids reduced by 33%
  - Active biomass ratio increased from 11% to 25%

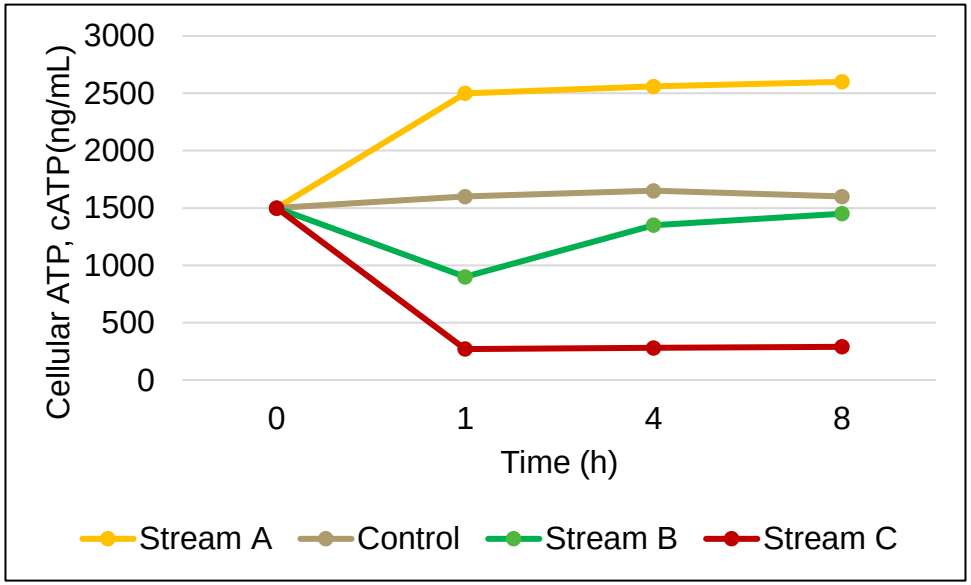




Toxicity Monitoring

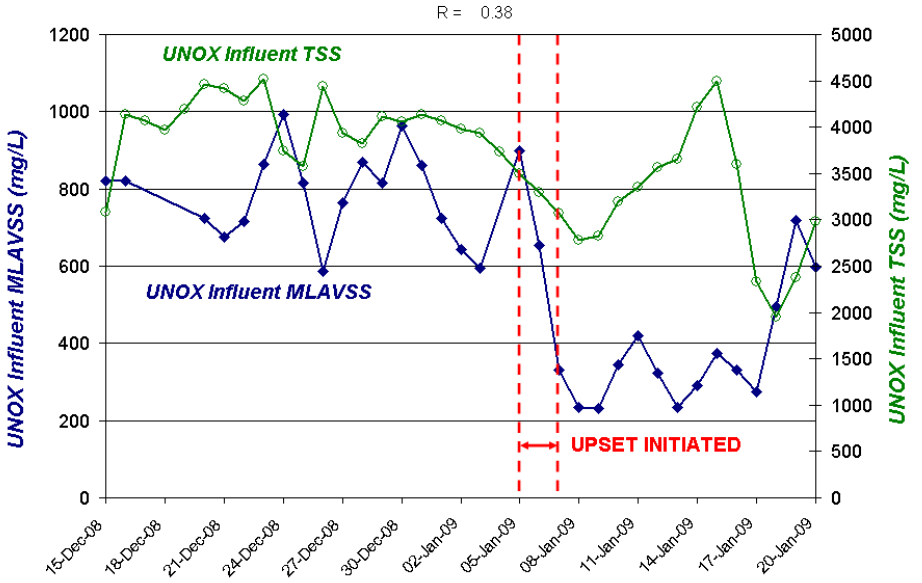
- Microorganisms are susceptible to inhibition from toxic materials.
- When something like this enters the facility, the organisms can become stressed and die.
- By mixing some influent and activated sludge, we can determine the affect each stream has on the organisms.

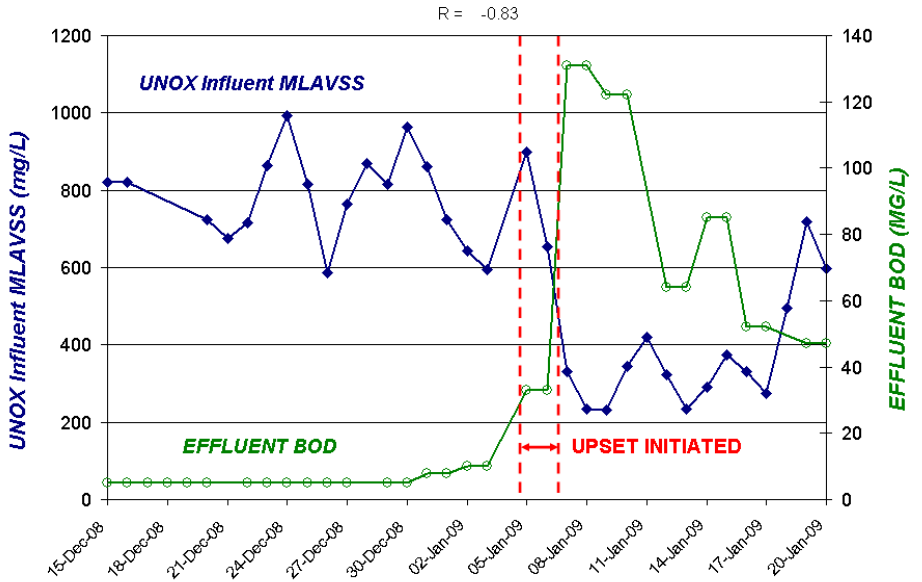




Not So Nice Surprises

- A plant received waste from ~10 industrial clients and was surprised with toxic upsets without warning.
- Following January 5th the plant saw:
  - 57% decrease in Living Biomass
  - 21% decrease in TSS
  - >200% increase of BOD

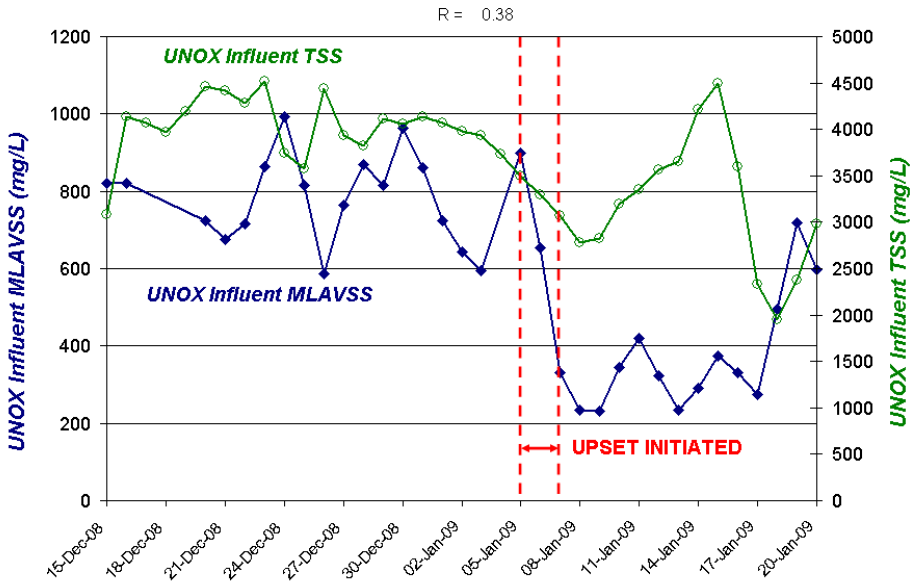




LUMINULTRA microbial monitoring

More Solids ≠ More Organisms

- Once the toxic event was identified, the bioreactor was reseeded to make up for lost organisms.
- Although the reseeding increased the TSS, the living biomass quickly fell.
- After attempting to reseed twice, it was determined the toxicity was entrenched in the sludge.



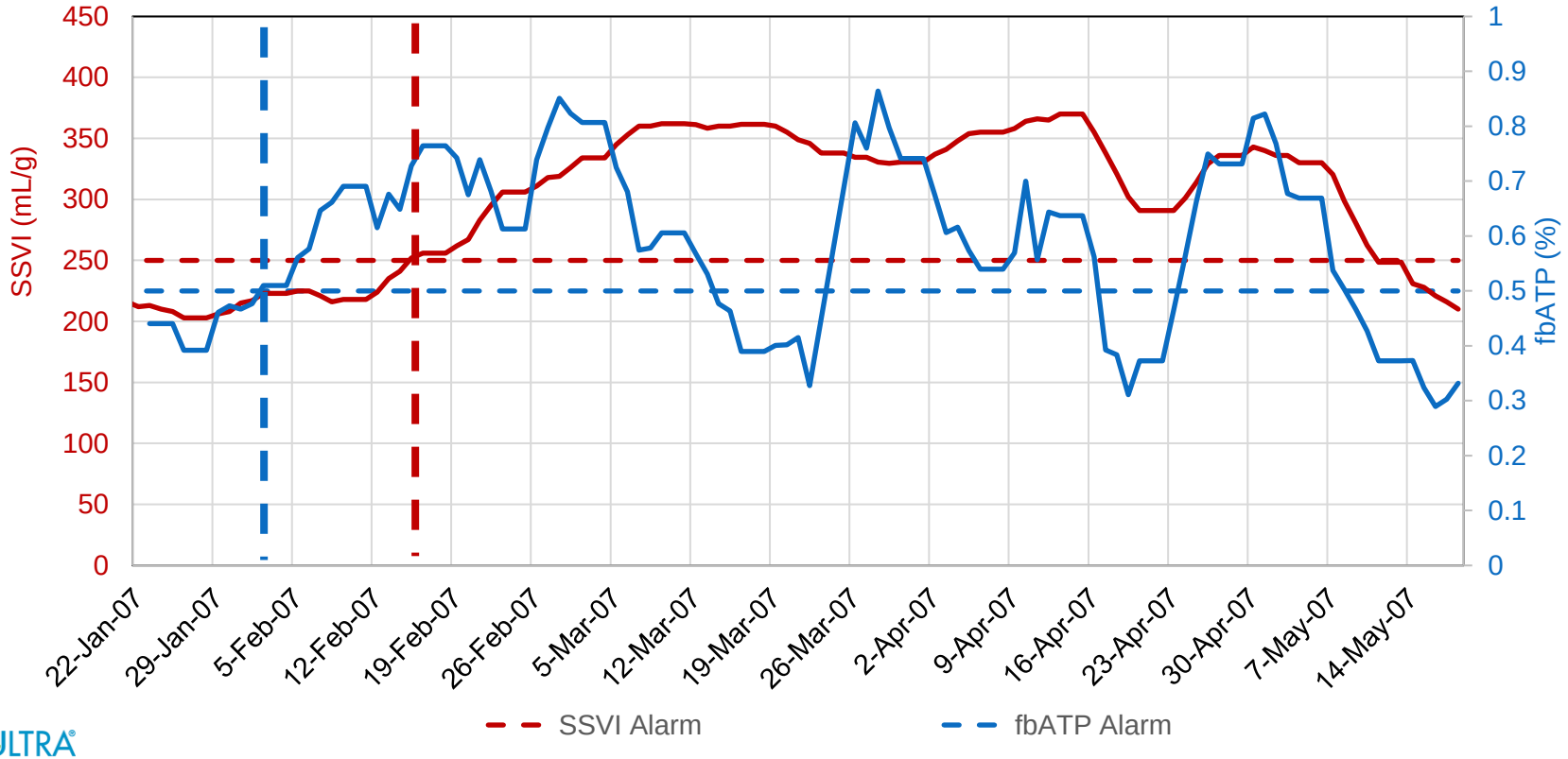
Filamentous Organisms

- Filamentous organisms are an important part of the settleability of the sludge blanket.
- However if too many filamentous organisms grow, it can lead to bulking and eventually carry-over.
- Excessive Filamentous growth can be cause by:
  - Influent fats/lipids
  - Poor O2 Transfer Efficiency
  - Low F/M

Floc Bulking Event

- A facility treating waste from a nearby pulp and paper mill experienced frequent floc bulking events.

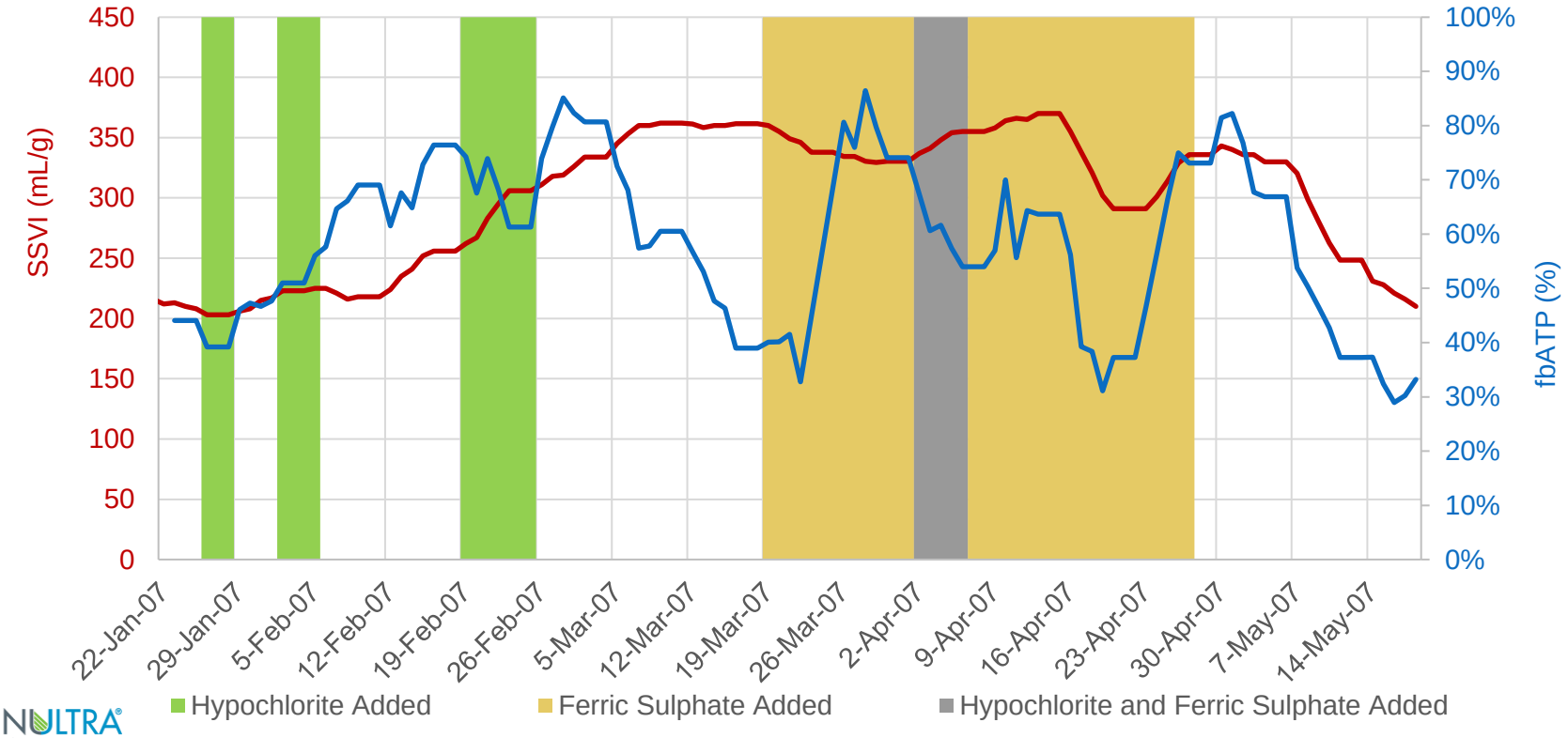
- A floc bulking event was identified when:
- SSVI > 250ml/g (February 12th)
- % fbATP > 50% (January 29th)



LUMINULTRA microbial monitoring

Floc Bulking Event

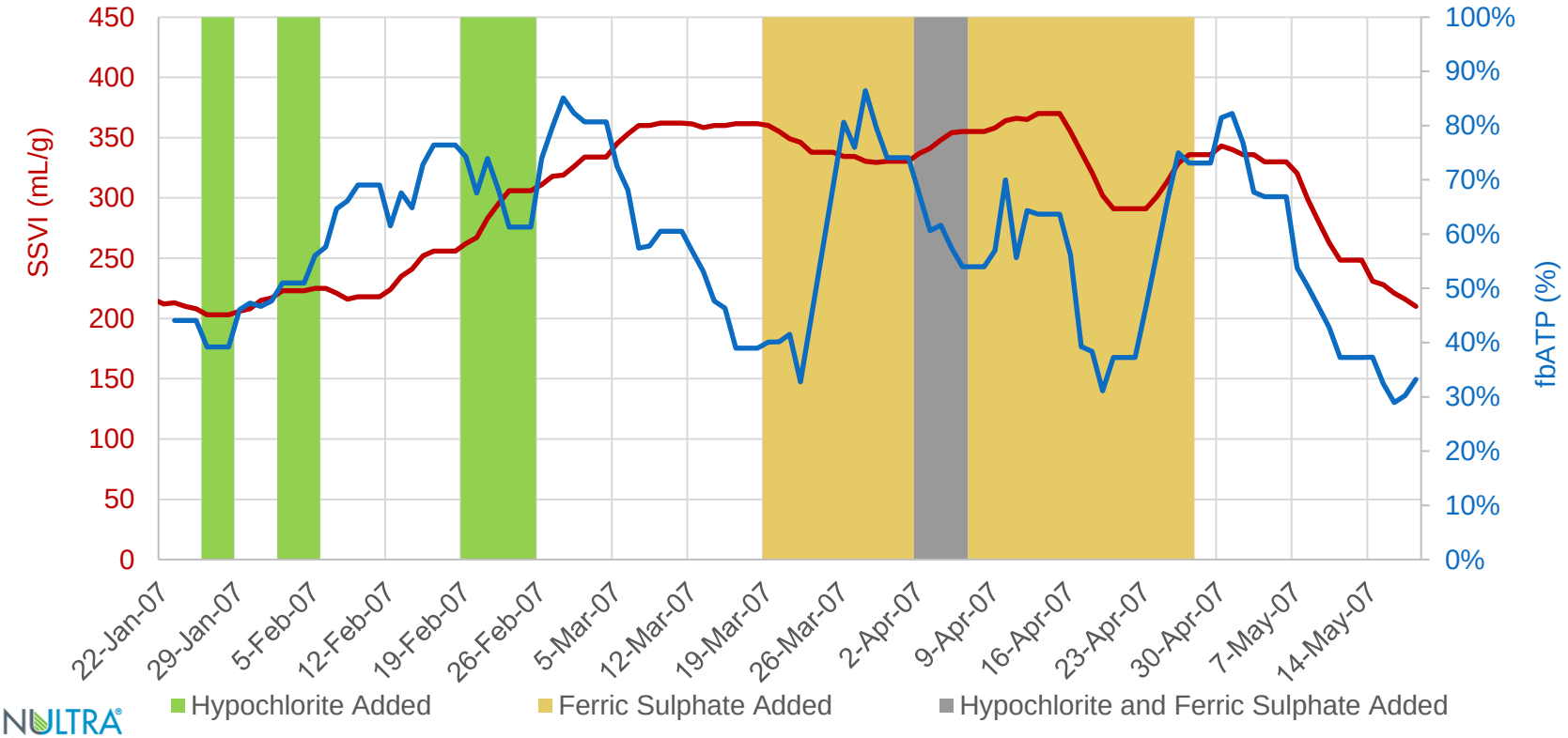
- A chemical treatment plan was conducted using Ferric Sulphate to assist in settling and Hypochlorite to destroy the filamentous organisms.
- When the Hypochlorite was removed, the filamentous organisms increased dramatically.



LUMINULTRA microbial monitoring

Floc Bulking Event

- The issue was determined to be an increased in volatile fatty acids entering the facility from the pulp and paper mill.
- Once the root cause of the issue was resolved, the levels returned to normal.

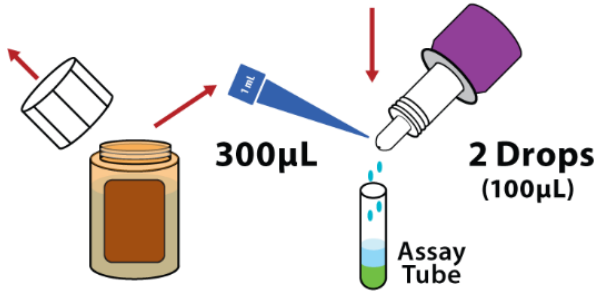


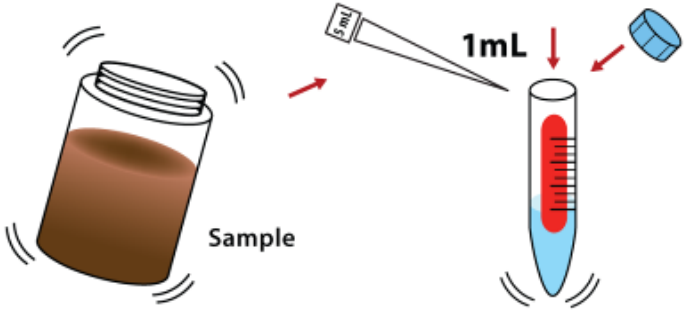
LUMINULTRA microbial monitoring

How to Take a Test – Clean Water

- As mentioned earlier, the test is broken into tATP and dATP.
- For both measurements only have to complete 1 calibration.
- Mix the calibration fluid with the firefly enzyme and place into a luminometer to take a reading.

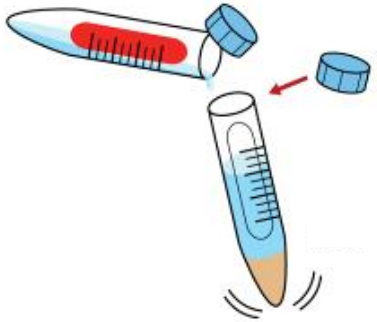
- <u>For the tATP test:</u>
- Invert the sample to ensure the organisms are well dispersed.
- Placed 1mL of sample into an extraction tube.

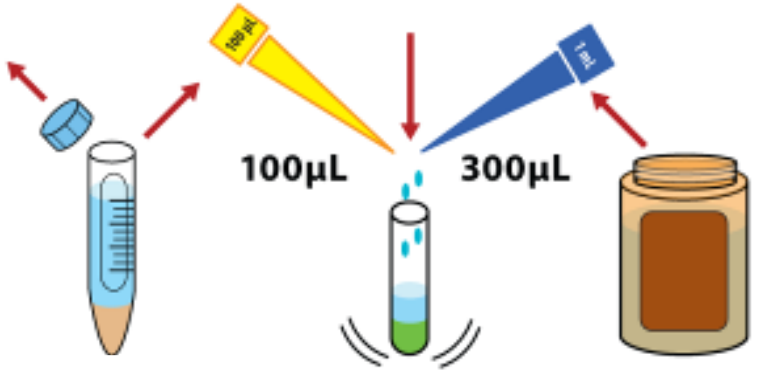




How to Take a Test – Clean Water

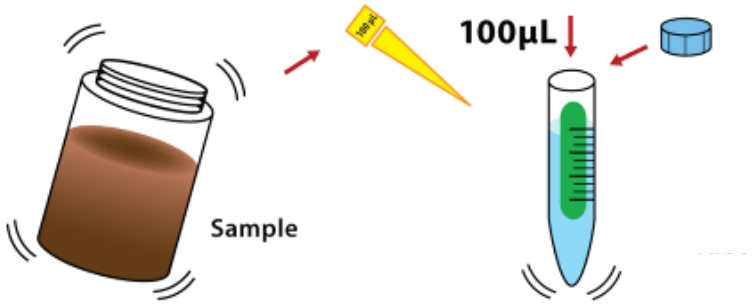
- Incubate for 1 minute.
- Then pour the extraction tube contents into a dilution tube that contains resign beads.
- Once the beads settle, the solution can then be mixed with the firefly enzyme.
- Place the reaction into the luminometer to take a reading.

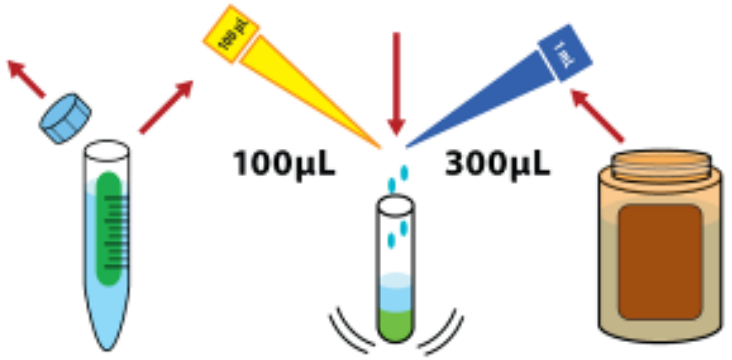




How to Take a Test – Clean Water

- <u>For the dATP test</u>:
- Invert the sample to ensure the organisms are well dispersed.
- Placed 1mL of sample into the stabilizer tube.
- Incubate for one minute.
- Mix some of the stabilized fluid with the firefly enzyme.
- Place the reaction into the luminometer to take a reading.





Summary – What is ATP?

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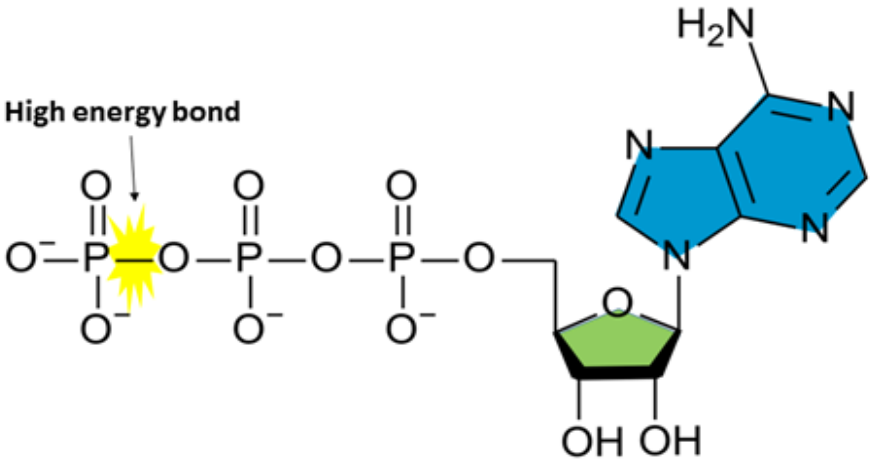
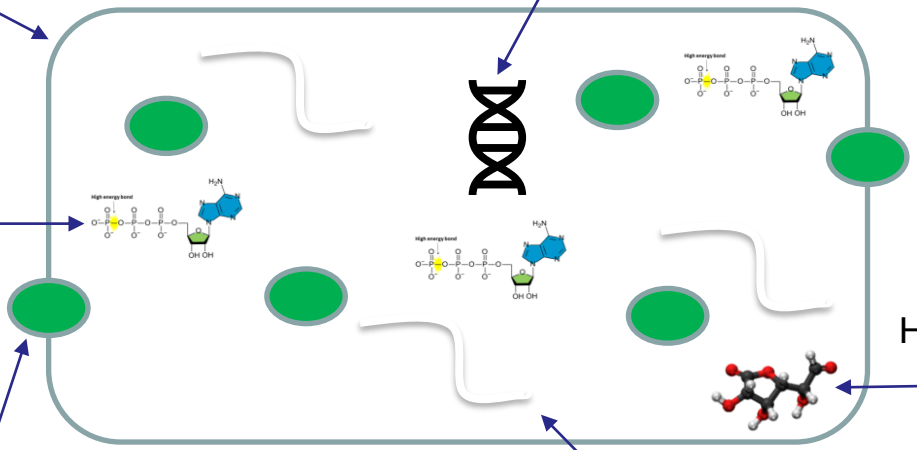


Why Was It Chosen?

DNA
Highly Specific and Readily Targetable

Membrane (Lipid)
Typically Not Specific



ATP Molecule
Energy Carrier Non-Specific

Metabolite
Hard to Find Specific Targets

Protein/Enzyme
Can be Specific But Need Antibodies or Other Recognition Molecules

RNA
Specific but Difficult to Handle

Adding a New Tool

Drinking Water

- Physical
  - Turbidity
  - Color
  - Alkalinity
- Chemical
  - Disinfection Residual
  - pH
  - Hardness
- Microbiological
  - Plate Counts☆
  - ATP

Water Resource Recovery

- Physical
  - Settleability
  - Total Suspended Solids
  - Temperature
- Chemical
  - BOD☆
  - Nitrogen
  - Phosphorous
- Microbiological
  - Microscope
  - DNA☆
  - Plate Counts☆
  - ATP

ATP in the Water Sector – Summary

Drinking Water

- Found the source of high biological risk.
- Determined if a turbidity event was biological.
- Compared disinfection to biological activity.
- Determined the effectiveness of a flushing activity.

Water Resource Recovery

- Looked at measuring the biological activity of activated sludge.
- Saw a facility keep the living population while decreasing the overall solids.
- Determined the effectiveness of a reseeding following a toxic event.
- Identified a floc bulking event and assessed the mitigation tactics.

Questions?

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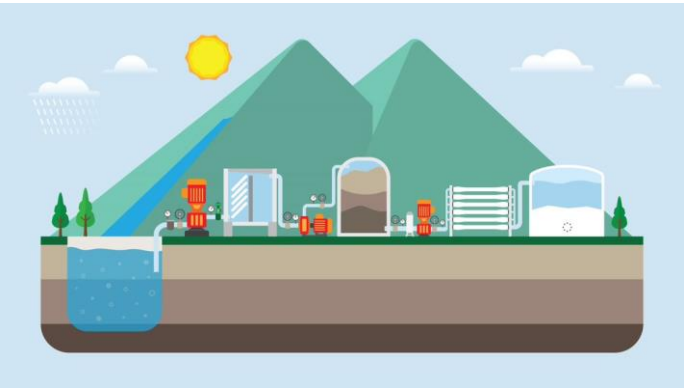

LUMINULTRA®
microbial monitoring

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