

Be Right[™]

How and Why to Monitor ATP for Biological Growth

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Agenda

- Overview of the Hach Company
- Hach and LuminUltra
- What is ATP
- Options for testing
- What is the value of knowing the microbial load
- Advance testing from LuminUltra
- Process ATP testing is EZ



Overview of Hach Company

- Began in 1947 in Ames, IA
- 1999: Acquired by Danaher Corporation
- Manufacturer of industrial and municipal water analysis solutions
 - process and laboratory instruments
 - chemistries
 - service and software
- Innovation leader
 - 527 patents, 130 patent families
 - strong investment in R&D and acquired technologies

OUR MISSION

Ensure water quality for people around the world.

OUR VISION

We make water analysis better—faster, simpler, greener and more informative—via unsurpassed customer partnerships, the most knowledgeable experts, and reliable, easy-to-use products.





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Overview of Hach Company

- 70⁺ RSM's
 - Regional Sales Managers
- 25⁺ CAM KAM ADM TSS CSS
 - CAM Corporate Account Managers
 - KAM Key Account Managers
 - ADM Application Development Managers
 - TSS Technical Sales Specialist
 - CSS Complex Sales Specialist
- 100⁺ Field Service Technicians
 - Field Service Partnerships
 - Startups, Commissioning and Training
- 41⁺ Technical Support Representatives
 - Global Customer Support
- 12⁺ Bench Service Technicians
 - Two service centers for repair and certification







Hach and LuminUltra Teaming Up



May of 2013

- Hach is a non-exclusive distributor of the LuminUltra Product Line
- Tech Support will be prepared to answer questions related to the following
 - Pre-Sale questions related to LuminUltra product offering
 - Ability to identify customers that may be interested in ATP testing
 - Ability to specify the correct LuminUltra test kits for different applications
 - Post-Sale troubleshooting of LuminUltra product offering
 - Exhibit elite level of support, ownership, and product knowledge LuminUltra product line

HACH[®] Be Right[™]

London

Paris

Heerenveen

LuminUltra offices spread across the globe

3.5K

Customers from around the world leverage LuminUltra's testing solutions

80+

25

OUPONT>

Countries with customers that rely on LuminUltra's products and services

Years of exceptional

production reliability

ECSLAB[®]

and innovation

\$345M

In customer value delivered to date

500K

Clinical COVID-19 testing reagents per week being supplied to Canada

Canada

Melbourne

About LuminUltra

Fredericton

Baltimore

Founded in 1995, LuminUltra is a biological diagnostic testing company headquartered in Canada with operations in 6 countries. It is widely recognized globally as a leader in developing tests and reagents for environmental, industrial, and diagnostic monitoring and is a key supplier of COVID-19 clinical testing reagents to the Government of Canada. Customers in over 80 countries trust LuminUltra's technology, production reliability and history of customer service excellence to deliver their essential services in a safe state.

LuminUltra proudly serves some of the top companies in the world including:



SUez



The Water Quality Toolbox

- Many physical and chemical parameters can be determined in minutes (or instantly via online analyzers).
- The technology has not existed to allow microbiological analyses to be done in the field and produce results quickly.

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 This results in significant limitations in water quality control capabilities.







The Problem

Color

LUMINULTRA

- Several Hach products give water testers the ability to *quickly* and easily assess several water quality parameters, such as:
 - Temperature • Turbidity
 - pН
 - Alkalinity TDS •

But what about *quick assessments* about *biological activity*? Options are



Company Confidering limited.

What is ATP?

- ATP = Adenosine Triphosphate
 - Primary energy carrier for all life.
 - Measurement takes only minutes.
- Since ATP is present in ALL living cells, quantifying it enables you to detect the entire population rather than just culturable cells.
- ATP testing has been used in food & medical hygiene for decades.
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The Basics of ATP Monitoring

- **ATP** = **A**denosine **T**ri**p**hosphate
 - Primary energy carrier for all life.
 - Measurement takes only minutes.
- LuminUltra is the first and only company to provide 2nd Gen.
 technology designed for fluid systems (far more sensitive, robust and reliable).





Industry Standard – HPC

- HPC's incubate for 24-48 hours.
- HPC's detect only ~0.1-1% of bioburden.
- Information is only provided on organisms that can grow...
 - ...in the media used;
 - ...at the temperature provided;
 - ...within the incubation time allowed.
- Regulatory parameters (e.g. Total Coliforms, E. Coli) measure a even smaller fraction.
- Many problem microorganisms are missed!





Strategy: Traditional Culture Tests



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Strategy: In-field ATP testing



Point-and-shoot strategy can be applied to isolate the root cause and solve problems on-the-spot.

What are the results of uncontrolled microbial growth?

- Biofilm accumulation
- Elevated disinfectant demand
- Taste & odor, cosmetic issues
- Non-compliance
- Premature infrastructure degradation (i.e. MIC)

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Which of these can be managed using compliance tests?

Limitations of Traditional Microbiological Testing

- None of the problems discussed on the previous slides are traced to a single type of organism – they are caused by the community as a whole.
- Attempts at total microbial content (e.g. HPC) are slow and a poor representation of the total population.
- The tools being used are not appropriate for the objective!

"90-99.9% of the bacteria found in water systems are not culturable in common laboratory media."

Risenfeld, 2004



What are the costs of uncontrolled microbial growth?

- Clean ≠ Compliant! Significant costs are incurred as a result of general growth.
- Several hours and large volumes of water required for flushing programs. Adds up to >\$122 US/hour to flush. Assumptions:
 - Water value = \$2 US/1000 USgal

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- Hydrant flow rate = 75 USgpm
- \$1/person/day for boil water advisory (conservative estimate).

Costs of Line Breaks

Location	Miles of Pipe	Total # of Breaks	Cost to Manage Annual Breaks ¹	
Phoenix	5,400	1350	\$4,050,000	
Dallas	4,600	1150	\$3,450,000	
Kansas City	1,912	478	\$1,434,000	
Macon	1,400	350	\$1,050,000	

• Average line break frequency:

23-27 breaks/100 miles of pipe/year (Kirmeyer et al)

• Direct costs to repair a break: \$3000

(American Water Works Service Company)

Costs of Line Breaks

Location	Miles of Pipe	Total # of Breaks	Cost to Manage Annual Breaks ¹	Potential Savings
Phoenix	5,400	1350	\$4,050,000	\$405,000
Dallas	4,600	1150	\$3,450,000	\$345,000
Kansas City	1,912	478	\$1,434,000	\$143,400
Macon	1,400	350	\$1,050,000	\$105,000

- Assume 50% of breaks due to corrosion (the balance is due to • 2020 Lumin COnstruction incidents, etc.)
 - Also (conservatively) assume that enhanced control reduces field service time by 10%.
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As a result...

- People have learned to live without microbiological data.
 - "We don't do micro. That's for the people across town/state lab/etc."
 - "We just keep our chlorine high."
 - "We just look at Total Coliform/E.Coli. We don't really care about other stuff."
- Many are aware of limitations but aren't aware that a solution exists.

As a result...

- If a problem is detected using traditional microbiological tools, it has been there for at least a couple of days.
- Since the time of sample collection, the problem may (and likely is) worse and spread downstream.
- What would it be worth to you to know about the problem immediately?



The Solution



- Value Proposition A rapid, non-specific measure of living microorganisms in water (or any other fluid sample).
- Four main advantages of LuminUltra's tests:
 - 1. Real-time feedback (< 5 minutes)
 - 2. Complete results (100% of species detected).
 - 3. Field-ready
 - 4. Test Kits designed for specific applications
- Decisions can be made on-the-spot, enabling *same-shift troubleshooting*.



PRODUCT OVERVIEW

2nd Generation ATP

"The first line of defence"

- Trusted solution for water-related industries for 15+ years;
- Rapid measure of total bioburden in < 5 minutes using a simple and portable test protocol;
- Facilitates timely risk assessments to identify areas of concern;
- Drives immediate action (i.e. more specific assays and/or adjustments to treatment strategies).







<u>Non-Filterable Samples:</u> cATP (Cellular ATP) = tATP (Total ATP) - dATP (Dissolved ATP)





Product Offering



- Luminometer-PhotonMaster
 - Powered via USB to laptop or desktop computer
- Application Specific Test Kits
 - Quench-Gone Aqueous (QGA)
 - Quench-Gone Organic Modified (QGO-M)
 - QuenchGone21 Wastewater (QG21W)
 - QuenchGone21 Industrial (QG21I)
 - QuenchGone21 Specialty (QG21S)
 - DSA Test Kit
- LumiCalc Software
 - Significant time savings in the organization, analysis, and reporting of data – spend minutes to achieve what took hours with traditional method.
 - Get interpretation as results are collected to make faster and better decision.

Company Confiderent connection to PhotonMaster saves time tabulating results and protects your data







Hach plus LuminUltra











QGA Test Kit Contents & Storage Conditions

Component (LuminUltra P/N)	Storage	Shelf Life
Luminase [™] Enzyme & Buffer Vials (Lu-3mL-FD) Luciferase Enzyme Reagent, 3mL	4 to 25℃	6 to 12 mo*
UltraCheck ™1 Dropper Bottle (UC1-5mL) 1 ng ATP/mL Standard, 5mL	4 to 25℃	18 mo
UltraLyse [™] 7 Bottle (UL7-125mL) ATP Extraction Reagent, 125mL	4 to 25℃	18 mo
UltraLute [™] (Dilution) Tube, 9mL (ULu-9mL-50R) ATP Dilution Reagent, 9mL	4 to 25℃	18 mo
Quench-Gone Syringe Filters, 25/pk (DIS-SFQG-25)	-	-
60mL Syringe, PP/Neoprene, 25/pk (DIS-S60-25)	-	-
100 to 1000µL Blue Pipet Tips, 100/rack (DIS-PT1-100R)	-	-
10 to 200µL Yellow Pipet Tips, 96/rack (DIS-PT01-96R)	-	-
12x55mm Test Tubes, 50/pk (DIS-CT12-50)	-	-



QGA – Quench-Gone Aqueous. Use for water samples whether fresh or brackish from and HACH

source when microbial control is required.

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QGA Quick-Reference Guide

Step 2 - Cellular ATP (cATP™) Analysis

2.1 – MEASURE SAMPLE VOLUME Determine volume and filter sample.

Sample Type	Volume (mL)
Cooling or Process Water	10 to 25
Fresh Brackish & Salt Water	25 to 50
Reclaimed Water, Effluents	25 to 50
Drinking and Sanitary Water	50 to 100
High Purity Water	> 100



2.2 – FILTRATION Filter sample.



2.3 – EXTRACTION Extract ATP from filter & dilute.



2.4 – ASSAY Measure ATP concentration.



NOTE: If RLU_{GATP} ≤ 10 using a PhotonMaster or Lumitester C-110, you are below the low- detection limit

NOTE: If RLU_{cATP} < 50 using a PhotonMaster or Lumitester C-110, consider accounting for background (RLU_{bg}). See Test Kit Instructions for guidance.









QG21W Test Kit Contents & Storage Conditions



Component (Part Number)	Store At	Shelf Life
Luminase ^{™W} Enzyme & Buffer Vials (LuW-3mL-FD) Luciferase Enzyme Reagent, 3mL	4 to 25℃	6 to 12 mo*
UltraCheck [™] 1 Dropper Bottle (UC1-5mL) 1 ng ATP/mL Standard, 5mL	4 to 25⁰C	18 mo
UltraLyse ™ 30 ²¹ (Extraction) Tube, 2mL (UL30(21)-2mL-50R) tATP Extraction Reagent, 2mL	4 to 25℃	18 mo
UltraLute™/Resin (Dilution) Tube, 8mL (ULuR-8mL-50R) tATP Dilution Reagent, 8mL	4 to 25℃	18 mo
LumiSolve [™] (Stabilizer) Tube, 10mL (LS-10mL-50R) dATP Stabilizing Reagent, 10mL	4 to 25⁰C	18 mo
UltraLyse [™] 30 ²¹ (Extraction) Tube, 10mL (UL30(21)-10mL-25R) ** tATP Extraction Reagent, 10mL	4 to 25℃	18 mo
100 to 1000µL Blue Pipet Tips, 100/rack (DIS-PT1-100R)	-	-
100 to 1000µL Wide-Mouth Pipet Tips, 100/rack (DIS-PT1WM-100R)	-	-
10 to 200µL Yellow Pipet Tips, 96/rack (DIS-PT01-96R)	-	-
12x55mm Assay Tubes, 50/pk (DIS-CT12-50)	-	-
2" x 2" 250µm Mesh Squares, 25/pk ** (DIS-MESH-25)	-	-
1.5" x 1.5" Weigh Boat, 25/pk ** (DIS-WD-25)	-	-
Scissor-Type Forceps, 1/pk** (EQP-FOR)	-	-

QG21W – QuenchGone21 Wastewater. Designed for quantification of biomass in wastewater treatment bioreactors, influents, and effluents. Also used for fermentation.

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NOTE: Kit uses LuminaseW

Luminase

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QG21W Quick-Reference Guide

Step 1 - UltraCheck[™] 1 Calibration

Perform one UltraCheck 1 calibration per day or per each set of samples analyzed.



Step 2 - Total ATP (tATP[™]) Included in QG21W[™] and QG21Wa[™] test kits.

2.1 - EXTRACTION Add sample to extract ATP.



2.2 - DILUTION Dilute out interferences.



2.3 - ASSAY Measure ATP concentration.



NOTE: If RLU_{IATP} ≤ 10 using a PhotonMaster or Lumitester C-110, you are below the low- detection limit.

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NOTE: If RLU_{ATP1} ≤ 500 using a PhotonMaster or Lumitester C-110, rehydrate a new bottle of Luminase^w for maximum sensitivity.

Step 3 – Dissolved ATP (dATP™) Included in QG21W[™] and QG21Wa[™] test kits.

3.1 - DILUTION Add sample to recover ATP.



3.2 - ASSAY Measure ATP concentration.



NOTE: If RLU_{dATP} ≤ 10 using a PhotonMaster or Lumitester C-110, you are below the low- detection limit.

STEP 4a – Floc Bulking ATP (fbATP™) Included in QG21Wa[™] test kit only.

4a.1 - FILTRATION Filter sample to separate bulking floc.



4a.2 - EXTRACTION Extract ATP from mesh.



4a.3 - DILUTION Dilute out interferences.



4a.4 - ASSAY Measure ATP concentrations.



NOTE: If RLU_{fbarp} ≤ 10 using a PhotonMaster or Lumitester C-110, you are below the low-detection limit.

STEP 4b – Attached Growth ATP (agATP[™]) Included in QG21Wa[™] test kit only.

4a.1 - EXTRACTION Extract ATP from sample.





4b.2 - DILUTION Dilute out interferences.



4b.3 - ASSAY Measure ATP concentrations.



NOTE: If RLU_{spATP} ≤ 10 using a PhotonMaster or Lumitester C-110, you are below the low-detection limit.

Municipal Water Management: Actions driven by ATP test results

- Field-deployable test format enables user to trace up the line to find the source of issues;
- Optimize flushing time/water usage;
- Boost disinfectant/burnout;
- Conduct system audits to optimize secondary microbiological monitoring tools to speciate.



Focus: Storage Tank Surveillance

- Storage tanks are often the first point at which regrowth becomes a problem.
- Whether it be due to long water age, stagnation, or infiltration, stored water represents a threat to downstream water quality.

[ATP]outlet > [ATP]inlet?

• If ATP leaving the tank is greater than that entering the tank then growth is occurring in the tank.

Focus: Storage Tank Surveillance

- Water quality issues had been apparent through a water delivery line with the source suspected to be a storage tank on-site.
- Samples were drawn at the source, the tank inlet, and the tank outlet.
- ATP tests were done at each location over a 3-day period.



Focus: Storage Tank Surveillance



Focus: Distribution System

- Diagnose water quality issues as biological or not in minutes;
- 2. Trace the line to the source;
- **3**. Apply corrective action;
- 4. Validate actions on-the-spot.

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- A utility performed a distribution system audit to compare microbiological contamination levels with total chlorine residual.
- To recap: ATP targets for drinking water systems are as follows:

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High Risk: >10pg/mL

Moderate: 1 < ATP < 10

Low Risk: < 1pg/mL Company Confidential

- As expected, ATP increased and total chlorine levels decreased with distance from the plant.
- BUT, in some cases ATP results persisted at elevated levels even in areas of adequately high chlorine.

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- Chlorine was boosted in addition to a flushing cycle.
- Chlorine residuals increased as expected with the highest levels being nearest to the plant.
- After the flush, same levels of residual Cl were observed at all points.

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- Despite the re-establishment of adequately high Chlorine, high ATP was still measured at the far ends of the distribution system.
- Localized focus could be placed in this area, whether it be in the form
 2020 Lumi Of additional flushing or additional root cause analysis.

■ Plant ■ West 4 ■ West 5 ■ West 6 ■ West 7 ■ West End

Case Study: Flushing

ATP levels dropped significantly after only 30 minutes to below the target 1 pg ATP/ml.

Savings: 2 man hours + 45,000 USgal of water.

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Case Study: Flushing

In this case ATP confirmed that the high turbidity was a non-biological issue and flagged the end of the flushing cycle after 30 minutes.

Savings: 3 man hours + 67,500 USgal of water.

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Focus: Nitrification

- Controlling nitrification requires a delicate balancing act:
 - $NH_3/Cl/pH$ all a function of what's in the water, difficult to make sure it's all as it should be.
 - Nitrifying bacteria cannot be detected using HPCs.
 - HPCs detect Denitrifying bacteria, but by that time, it's already too late.

Summary on ATP Testing

- ATP technology overcomes the limitations of traditional microbiology by taking microbiological testing into the field and producing results as quickly as other water quality parameters;
- Reveal, isolate and solve water quality problems in a fraction of the time that would otherwise be needed;
- Use results of in-field ATP tests to drive and prioritize additional testing to further characterize water quality.

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PRODUCT OVERVIEW

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GeneCount™ qPCR

Isolates specific microbes

- qPCR testing has historically been complex, expensive, and a significant investment that only research labs would pursue.
- GeneCount is very "on brand" with LuminUltra's traditional approach: practical and portable yet refusing to sacrifice accuracy for the sake of simplicity.
- Value Proposition: Test for the specific threats that concern you most without involving a lab, thereby saving significant time and money.
- Expandable platform to include numerous other tests in the future, including infectious diseases in addition to more pathogens and troublesome microbes.

PRODUCT OVERVIEW

GeneCount[™] qPCR

Additional Assays:

- Total Prokaryote
- Sulfate-Reducing Prokaryote
- Sulfur-Oxidizing Prokaryote
- Methanogens
- Iron Reducing Bacteria
- Total Fungi
- Total E.Coli
- Legionella sp.
- NEW: SARS-CoV-2

Coming Soon:

- Legionella pneumophila
- Nitrifying Prokaryotes
- Nitrate-Reducing Bacteria
- Acid-Producing Bacteria

LUMINULTRA

Pseudomonas

Having a microbial safety plan is crucial to ongoing risk management

uminUltra's multifunctional GeneCount line provides both the platform and assays needed for ongoing pathogen defense!

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HACH

The EZ Series: online water analysis made easy

A Dynamic Process – Real Time Monitoring Needed

* Graph by Montana State University

The Objectives:

- "Healthy biofilms will release lumps of bacteria once fully grown: sessile bacteria turn planktonic again: this is a risk.
- When Biocides are dosed they will only touch the surface of the biofilm releasing similar lumps of bacteria: risk.
- A clean system can be obtained by: cleaning and monitoring of low concentrations planktonic bacteria.

Planktonic / Sessile / Planktonic

Cell dies and ATP is rapidly degraded

ATP serves as a reliable biomarker for living organisms in a sample

Things to know about measuring ATP

ATP can be used as a parameter in high-frequency, automatic, on-line microbial analysis in water Online options to measure Free ATP, Total ATP and Intracellular ATP

Proprietary Sample Pretreatment to Detect Living Organisms

What is the relevance of measuring the different ATP portions?

- Extracellular ATP or free ATP is the portion of ATP released by dead cells
- Total ATP is obtained after lysis of the biomass by sonication of the sample
- Intracellular ATP is the portion of ATP from the metabolically active (living) organisms

Intracellular ATP = Total ATP – Extracellular ATP

Bonus Material: Free ATP can be used in reverse osmosis (RO) applications to monitor contaminant removal

ATP chemiluminescence reaction

ATP assays using luciferin/luciferase reactions allow to assess microbial load in water sources. The ASTM D4012 (Standard Test Method for Adenosine Triphosphate Content of Microorganisms in Water) was developed as a quick and sensitive alternative to plate counting.

EZ7300 Series - General

What?

The first microbiology analyzer using the ATP firefly assay and complying with international standard method **ASTM D4012-81**

The on-line microbiology analyzer brings operators

- Fast results (few minutes ≈ real-time data)
- Automation
- Analytical performance
- Reliability

Hach EZ7300 Series ATP Analyzer

The first microbiology analyzer using the ATP firefly assay and complying with international standard method **ASTM D4012-81**.

- Fast results (few minutes ≈ real-time data)
 - 10 minutes can be delayed to 2.5 hours
- Analytical performance, LOD of 0.05 pg/mL
- Up to 8 sample streams possible
- Highly sensitive and stable ATP reagents
- Automation and cleaning
 - Rinsing after each sample.
 - Cleaning with HCl to break down any biofilm.
 - Cleaning with NaOH to hydrolyze remaining bacteria

EZ7300 Series - High analytical performance

- Complete ATP recovery: detection of intracellular, extracellular and total ATP
- Rapid measurement: 7 10 minutes (including lysis)
- No delay between sample take-off and measurement
- Smart features: automatic calibration and 3-step cleaning protocol
- Low limit of detection (LOD): 0.05 pg/mL (0.1 pM) ATP *
- Extended reagent stability (see further)
- Factory configured, tested and calibrated

* 0.05 pg ~ 50 *E. coli* sized bacteria

EZ7300 Series – Proprietary sample pretreatment

Question: what is one of the main challenges in measuring the microbial load (contamination) in water?

Answer: in order to have a clear picture of the microbial load it is important to differentiate ATP portions within living cells from non-living cells

ATP in the water source can be located...Either inside bacteria or other cells= intracellularOr freely in the water surrounding the cells

= extracellular or free ATP

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EZ7300 Series – Proprietary sample pretreatment

What is the relevance of measuring the different ATP portions?

- Extracellular ATP or free ATP is the portion of ATP released by dead cells
- Total ATP is obtained after lysis of the biomass by sonication of the sample
- Intracellular AP is the portion of ATP from the metabolically active (living) organisms

Or:

Intracellular ATP = Total ATP – Extracellular ATP

EZ7300 Series – Proprietary sample pretreatment

Solution: EZ Series proprietary sample pretreatment unit performs a controlled ultrasonic lysis of the sample which disrupts the living cells

- Sampling
- Addition luciferase and luciferin
- 1st light output correlates with free ATP
- Cell lysis with ultrasonic treatment
- Addition luciferase and luciferin
- 2nd light output correlates with total ATP

Real time monitoring of raw water intake and treated water

The objectives:

At the Alfred Merritt Smith Water Treatment Facility

- Obtain real-time information to further optimize the ozonation of the raw water coming from Lake Mead.
- Refine and optimize the Chlorine gas disinfection process used on the finished drinking water.

Saint Paul Regional Water Services' McCarron's Water Treatment Plant

Water Use (In Million Gallons) Average daily - (2016) 38.2

Area Supplied (In Square Miles) Saint Paul - 56.2 Suburbs - 66.6

Water Treatment Plant (In Million Gallons) Daily plant capacity – 144

Total treated, finished water - 88

Number of people served - 432,911

Optimization of a biological filter and plant risk mitigation

The objectives:

- Monitor in real time the microbial load of the influent and effluent of their biological filter.
- Determine the efficiency of the biological filter in removing biomass (microbial load)
- Trouble shoot/mitigate risk (corrosion) in other parts of the plant using the grab sample line of the analyzer.

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Field data: drinking water

The graph shown on the right is the statistical summary from analysis between June and September2017 regarding filter influent and effluent of a biological filter.

Filtration process is effective in removing ~57% of influent biomass based on the ATP data.

Data courtesy of Saint Paul Regional Water Services, MN, USA

Drinking water production company senj, vodovod južni ogranak senj

- Water source for production of drinking water is accumulation from two rivers (Lika and Gacka).
- HE Senj electricity power plant also use this accumulation for electricity production. Maintenance plan - 3 times a year.
- Maintenance plan affect on quality of raw water, especially on turbidity and microbiology

Solution

- In normal condition customer use sand filter
- In case of microbiology excess customer will use ATP analyzer as early alarm and switch purification to ultrafiltration

THANK YOU FOR YOUR TIME TODAY

