ATP measurements for online monitoring of microbial drinking water quality - evaluating potential

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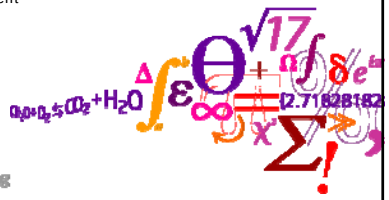
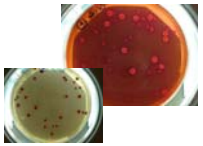
SENSOWAQ



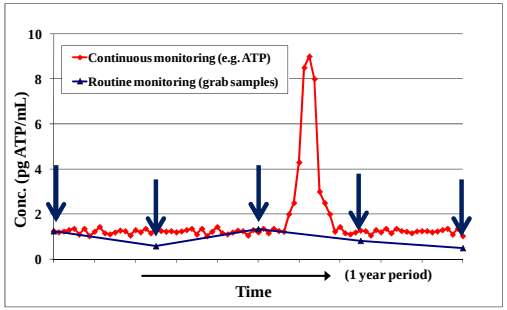
DTU Environment
Department of Environmental Engineering

Measuring microbial drinking water quality today?

- Current methods: late results (2 to 3 days)
  - → contamination spread in the distribution system
  - → water consumed
- Low frequency sampling
  - → contaminations not detected



Continuous monitoring – concept



- Detection of a pulse contamination through continuous monitoring between regulatory controls (grab sampling)

Sensor application

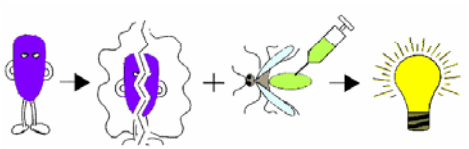
Possible contamination scenarios:	Monitoring points:
– Breach in hygienic barrier (treatment) at the water works	– Water works outlet
– Intrusion of contaminant (e.g. dirt, surface water) when renovating the distribution network	– Major pipe connections/branches
– Intrusion of contaminant (e.g. bird feces, surface water) into water tower/reservoir	– Water tower/reservoir outlet
– Sensitive consumers (e.g. hospitals, pharmaceutical and food industry)	– Inlet to consumer

'Novel' method

ATP for continuous monitoring of microbial drinking water quality???

- Advantages:
  - Simple measurement procedure
  - Results within minutes (real-time analysis)
  - Measurement for all active cells
  - Small sample volumes

Principle of ATP-method



firefly luciferase

$$ATP + D-luciferin + O_2 \xrightarrow{+\,Mg^{2+}} AMP + PP_i + oxyluciferin + CO_2 + light$$

1. Extraction of ATP (cell lysis)
2. Reaction with luciferine/luciferase (production of light)
3. Light emission measurement by a photomultiplier (relative light units)

Aim

- To optimize and further develop the ATP method for measuring microbial drinking water quality
  - Quantitative measurements on microbial ATP
  - Solving problems: matrix effects (e.g. turbidity, color) and internal standard
  - Stability and storage of reagents
  - Cell lysis techniques (detergents, heat)
  - Handling and storage of samples during analysis
- Effect of various contamination sources (waste water, surface water etc.) in different experimental set-ups
- Validation of method on a sensor platform (field investigations)

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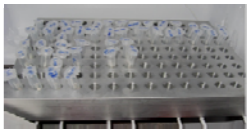
Background for lab experiments

- Sensor perspectives: maintenance e.g. once a month
- Focus on storage conditions and stability/activity of reagents

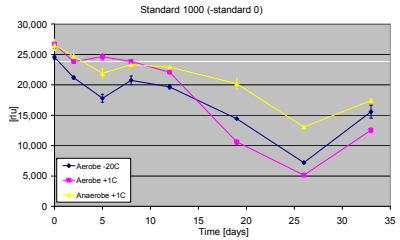


Activity of luciferine/luciferase (LL)

- How does activity of luciferine/luciferase reagent decrease with time?

Activity of luciferine/luciferase (LL)

- Fluorescence of LL decreased with time
- Reduction in activity (rlu) after 33 days:
  - Aerob -20ºC : ~40%
  - Aerob +1ºC : ~60%
  - Anaerob +1ºC : ~30%
- Same reduction was observed for all tested standards (10, 500 and 1000)



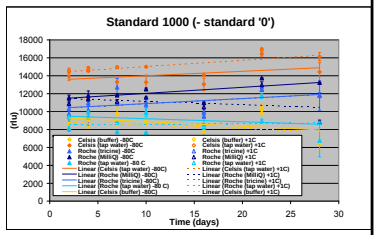
Activity of luciferine/luciferase (LL)

- Acceptable loss in activity of luciferine/luciferase
  - Calibration by addition of internal ATP standard (IS)

Stability of IS?

Stability of ATP standards

- Reduction in activity:
  - Roche standard in tap water: 24%
  - Roche in tricine: 15%
  - Roche in MilliQ water: 7%
  - Celsis in buffer: 13%



- Celsis standard in sterile filtrated autoclaved tap water was stable throughout a 4 week period at +1ºC

Cell lysis efficiency

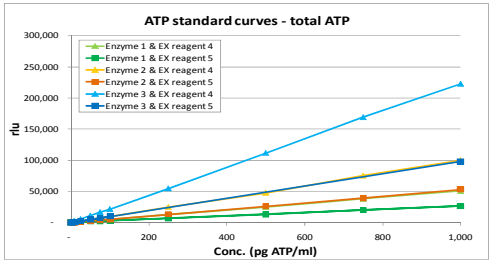
- Quantitative analysis for microbial ATP depends on an efficient cell lysis
- Complete lysation → high output and accurate measurement

- Challenges:
  - low quantities of ATP in drinking water
  - no loss of ATP after extraction
  - no significant inhibition on the luciferin/luciferase reaction

- Investigation of 9 commercial reagents in order to identify:
  - sensitivity
  - measurement stability
  - cells lysis efficiency

Commercial ATP reagent kits

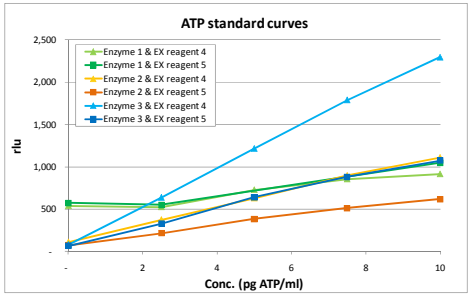
- **Promicol**: 3 enzymes reagents and 2 extraction reagents



- Rlu signal: Enzyme 3 > enzyme 2 > enzyme 1
- Different slopes (quenching of light signal): extraction reagent 5 gives a higher quenching than extraction reagent 4

Commercial ATP reagent kits

- Linearity and y-axis intersection:



- Enzyme 1 → lowest sensitivity

Commercial ATP reagent kits

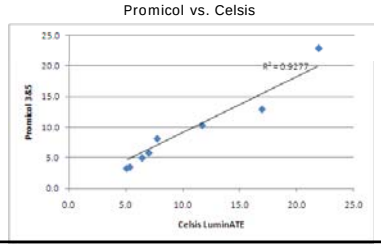
- Drinking water samples → microbial ATP

Sample ID	Enzyme 1		Enzyme 2		Enzyme 3	
	Extraction 4 (pg ATP/ml)	Extraction 5 (pg ATP/ml)	Extraction 4 (pg ATP/ml)	Extraction 5 (pg ATP/ml)	Extraction 4 (pg ATP/ml)	Extraction 5 (pg ATP/ml)
115	-3.7	-3.7	1.5	4.1	1.8	3.5
237	-1.1	-3.0	3.1	6.7	4.1	8.1
208	-3.6	-3.7	1.8	3.7	1.7	3.3
326	-4.0	-3.7	2.1	4.3	3.2	5.0
302	13.5	6.1	21.6	31.6	13.5	22.9
421	1.5	-2.1	6.3	15.1	6.4	12.9
Lyngby WW	0.6	-2.8	6.4	11.0	6.3	10.3
YE in DW (10^{0})	369.7	903.6	339.9	876.1	308.2	792.2
YE in DW (10^{-1})	28.1	61.8	36.3	61.0	33.8	56.3
YE in DW (10^{-2})	-4.8	-4.3	3.4	5.6	3.4	5.8

- Negative values indicate too low sensitivity (enzyme 1)
- Higher yield with extraction reagent 5 → a better cell lysis

Commercial ATP reagent kits

- Promicol:
  - Overall good measurement stability: CV <5% in most cases
  - Various sensitivties of enzymes: enzyme 3
  - Various cell lysis efficiency: extraction reagent 5
- 6 other reagent kits were also investigated



Performance of the ATP method

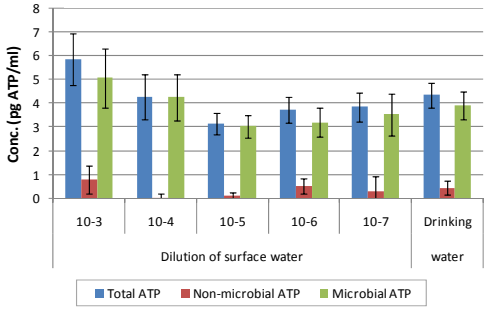
- Response in ATP in different contamination scenarios
  - surface water
  - waste water
- Reagent significance: LuminATE vs. RapiScreen Health (Celsis)

Simulation of drinking water contaminations

Surface water contamination

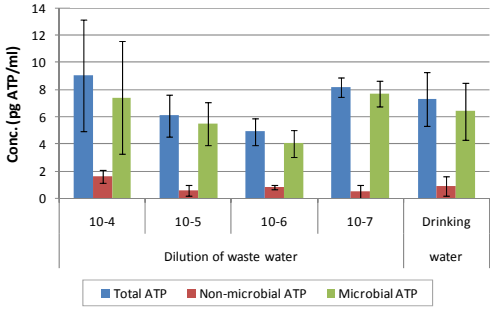
- Surface water was collected from dig-outs for pipe connections

Surface water contaminations
LuminATE reagent kit



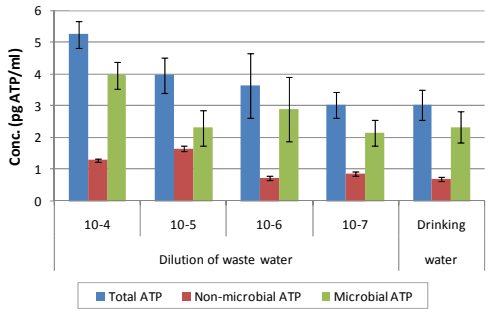
- 10^{-2} to10^{-3} dilution of surface water (1 to 10 L in 1 m^3 drinking water)

Waste water contaminations
LuminATE reagent kit



- 10^{-3} to 10^{-4} dilution of waste water (0.1 to 1 L in 1 m^3 drinking water)

Waste water contamination
RapiScreen Health reagent kit



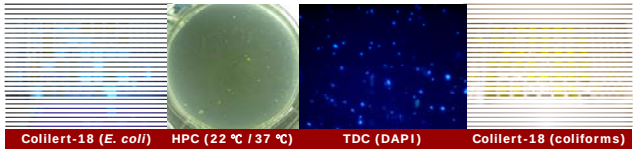
- 10^{-4} dilution of waste water (0.1 L in 1 m^3 drinking water)

Waste water contamination

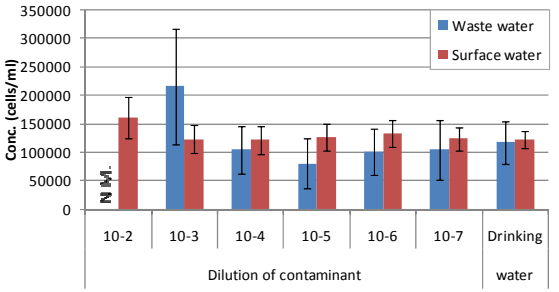
- RapiScreen Health vs. LuminATE
  - Improved quantification of low ATP-concentrations → especially fraction of non-microbial ATP
  - More stable measurements
  - Slightly improved sensitivity

Traditional microbiological methods

- ATP compared with other methods:
  - Total direct cell counts (DAPI)
  - Heterotrophic plate counts (yeast)
  - Colilert -18 (*E. coli* and coliforms)

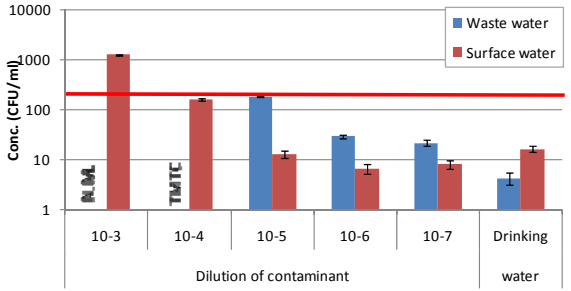


Drinking water contaminations
Total Direct Counts (DAPI)



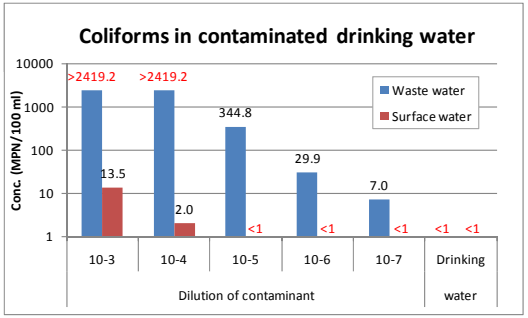
- Surface water:10 L in 1 m³ drinking water
- Waste water: 1 L in 1 m³ drinking water

Drinking water contaminations
Heterotrophic plate counts (yeast extract) 22°C



- Surface water:1 L in 1 m³ drinking water
- Waste water: 0.1 L in 1 m³ drinking water

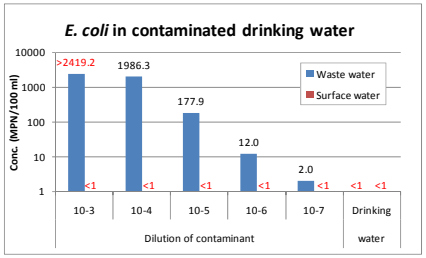
Drinking water contamination
Colilert-18 (coliforms)



- Surface water: 100 ml in 1 m³ drinking water
- Waste water: 0.1 ml in 1 m³ drinking water

Drinking water contaminations
Colilert-18 (*E. coli*)

- Colilert-18 (*E. coli*)



- Surface water: not detected (30 MPN/100 ml in surface water)
- Waste water: 0.1 ml in 1 m^3 drinking water

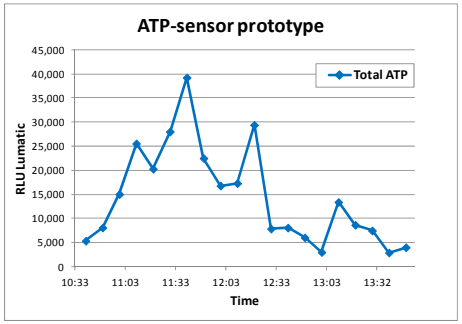
Conclusions

- ATP compared to other methods (TDC, HPC, Colilert-18)
  - HPC and Colilert-18 more sensitive
  - Depending on load of bacteria in contaminant
- RapiScreen Health was more sensitive than LuminATE
- ATP can be used as a monitoring method for microbial drinking water quality. Can detect
  - 10^{-3} – 10^{-4} dilutions of surface water in drinking water
  - 10^{-4} – 10^{-5} dilutions of waste water in drinking water
  - Continuous contamination at low concentrations → no
  - Pulse contaminations → yes

Field testing ATP-sensor prototype

- Collaboration with Promicol (The Netherlands)
- Field investigations
  - Continuous time series of ATP concentrations in drinking water → detection of pulse contaminations?
  - Fluctuations in ATP concentrations on short term basis (minutes to hours) and long term basis (days)
  - (In)consistency between standard microbial methods and ATP measurements (grab samples vs. continuous monitoring)

Continuous monitoring



- Peak around lunch time

Summary

- Criteria for ATP as a monitoring method
  - Luciferin/luciferase activity: ok
  - Stable internal ATP standard: yes
  - Lysis efficiency: depends on extraction reagent
  - Sensitivity: depends on enzyme reagent
- Performance of ATP as a monitoring method:
  - Detection of waste water and surface water: yes, depends on load
  - Continuous time series on a sensor platform: validation upcoming...

Thank you

- **Questions?**

...NOW!