



Development of an early warning system for water supply networks based on automated ATP monitoring

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A photograph of two workers in high-visibility safety gear. One worker in a yellow suit and helmet is operating a high-pressure water sprayer, creating a large mist of water. The other worker in an orange and black suit and helmet stands nearby, smiling. They are outdoors, with green foliage and a grey wall in the background.

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Abstract title: Development of an early warning system for water supply networks based on automated ATP monitoring

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Abstract text:

Drinking water systems are currently monitored for contamination by grab sampling and culture based analytical techniques that provide results within few days. To avoid health risks from exposure to contaminated water, there is a strong need to develop early warning systems that can detect a contamination in nearly real time. Adenosine TriPhosphate (ATP) is an energy carrier molecule present in all living cells and monitoring of its concentration in drinking water is a promising technique for two main reasons. Firstly, ATP is an indicator of total microbial activity, meaning that only active microorganisms are detected, and the detection is not restricted to a specific microbial type. Secondly, ATP analysis can provide results in few minutes, creating a great potential for real time monitoring. ATP is currently measured in the laboratory by commercial test kits. The aim of this work is to implement the currently existing ATP measuring protocols into an automated ATP measuring platform that can be installed at the drinking water treatment plants and in the distribution system for continuous and automated monitoring.

The developed system uses a microfluidic cartridge, where mixing of the samples with the reagents and detection of light from the bioluminescent assay are implemented. The assay takes place in two steps: ATP release from the cells using an ATP releasing reagent, and reaction with the Luciferin/Luciferase reagent producing light that is detected by a photomultiplier placed on top of the microfluidic cartridge.

Initially, we optimized the assay conditions using ATP standard solutions. The effect of temperature was investigated and highest signals were recorded at 25°C. The flowrate ratio of standard solution and Luciferin/Luciferase reagent was tested to minimize the volume of reagent volume used, and a 1 to 1 ratio was standardized for the assay.

ATP standard solutions were used to fit linear calibration curves for both free ATP (no ATP release step) and for total ATP (with release of ATP from the cells). The use of ATP releasing reagent however, caused the formation of particles that deposited in the microfluidic cartridge, ultimately clogging its channels. Particle formation was also observed in offline tests and was attributed to the mixing of the ATP releasing reagent with the Luciferin/Luciferase reagent. Further assay development focused therefore towards an alternative ATP releasing method.

Thermal ATP release from the cells was investigated by incorporating a heating/cooling step in the assay. The thermal lysis step was designed to be a flow-through step before the microfluidic cartridge that, in principle, does not need additional manual handling. The efficiency of the thermal ATP release was tested offline and compared to lysis by the ATP releasing reagent (chemical lysis) in different water types including tap water (treated groundwater) spiked with 20 pg/L ATP, untreated surface water, stored rainwater and a dilution of *E. coli*. The efficiency of the thermal lysis step was different for the different samples. Both chemical and thermal lysis showed consistent ATP release in surface water, but chemical lysis was more efficient in rainwater. On the other hand, thermal lysis proved to be more efficient in releasing ATP from the *E. coli* dilution, ultimately suggesting that the lysis efficiency of each method is dictated by the type of cells present in the sample.

Overall, the developed prototype system is a proof-of-concept for a lab-on-a-chip ATP analyzer. Particular focus in this part of the work was dedicated to optimize the assay conditions and the experimental protocol. This project is an ongoing work and further validation and optimization are supported by the EU granted project Aquavalens.

