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Towards the development of an automated ATP measuring platform to monitor microbial quality of drinking water

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Abstract: This work aimed to develop an automated and nearly on-line method to monitor ATP levels in drinking water as an indicator of microbial contamination. The system consists of a microfluidic cartridge installed in a light tight box, where the sample is mixed with the reagents and the emitted light is detected by a photomultiplier. Temperature in the assay box is controlled and set to 25°C. Calibration of the system using ATP standard solutions was successful, both for free and for total ATP. Chemical release of ATP by reagent addition however resulted in the formation of particles that ultimately clogged the microfluidic channels. An alternative thermal lysis step was implemented, by adding a flow-though heating/cooling step to the system. Thermal lysis showed efficient release of ATP from an E. coli dilution, but the releasing efficiency varied according to the type of water. Overall, the developed prototype system proves the concept of a lab-on-a-chip ATP analyzer.

Keywords: drinking water, contamination, monitoring, distribution system

Introduction

Contamination of drinking water by accidental ingress of wastewater or stormwater in the supply network is not a rare incident and can pose serious risks to public health. Currently, monitoring of the water supply system is typically done by grab sampling at selected locations, and analysis uses almost exclusively culture based methods that can only provide results in a couple of days. This time delay is a great challenge for the effective management of contamination cases, as it prolongs the consumers' exposure to the contaminated drinking water. There is therefore a great need for development of early warning systems that can signal a contamination case in nearly real time.

Adenosine TriPhosphate (ATP) is a coenzyme present in all living cells and is a microbial activity indicator that can be monitored in drinking water to identify sudden peaks that signal a contamination case. In this work, we aim to develop an automated ATP measuring platform to be installed at the drinking water treatment plants and in the distribution system for continuous monitoring.

Material and Methods

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; first ATP is released from the cells and then reacts with the Luciferin/Luciferase enzyme and its substrate to produce a light signal that is detected by a photomultiplier. Both steps of the assay occur within the same microfluidic unit, and the detected signal is transmitted and recorded by a PC. The photomultiplier is placed on top of the microfluidic cartridge in a light tight box (dimensions are 16×26 cm), so that only the light from the reaction is detected (Figure 1).

Results and Conclusions

Initially, we aimed to optimize the assay conditions using ATP standard solutions. The effect of temperature in the assay box on the recorded signal was investigated in the range between 25 and 32°C, and highest signals were recorded at 25 °C. Then the ratio of standard solution and reagent flowrate was tested to minimize the volume of reagent used. A positive correlation between higher reagent to standard solution ratio with signal was found, and the assay was standardized at a 1 to 1 ratio. Also hydraulic mixing in the microfluidic device was investigated in different microfluidic cartridge designs by colour tracer microscopic imaging.

The system was subsequently calibrated using ATP standard solutions that followed a linear trend both in a wide concentration range and in the low concentration range relevant for drinking water systems (Figure 2). Calibration curves were run with the same standards for both free ATP (no ATP release step) and for total ATP (with release of ATP from the cells). The ATP release step included reaction of the sample or the standard solution in the case of the calibration curves, with an ATP releasing reagent. Although no ATP release is necessary during calibration, the ATP release step was incorporated to reflect the complete assay protocol. 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 enzyme. 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 assay box 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 (Figure 3). 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 an 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.

Figures

Figure 1. External view of the light-tight box containing the microfluidic cartridge. The photomultiplier shown in the picture is placed perpendicular to the surface of the cartridge and the collected signal is transmitted to a PC.

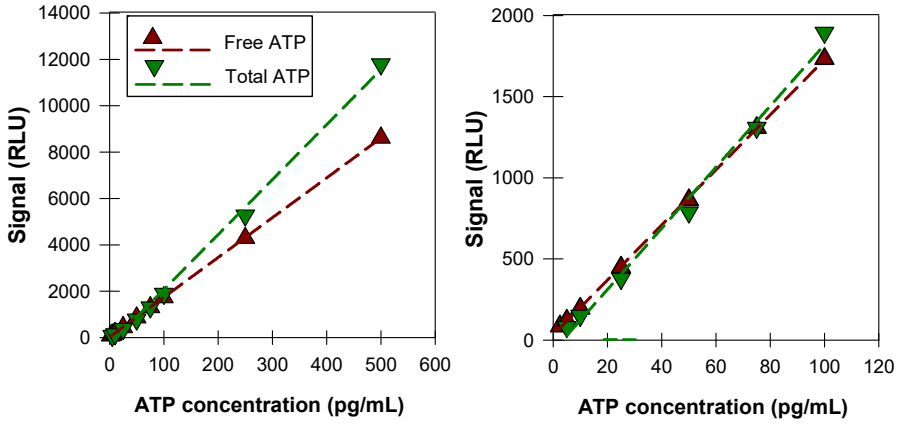

Figure 2. Calibration curves for free and total ATP for a wide (left panel) and low ATP concentration range relevant for drinking water systems (right panel).

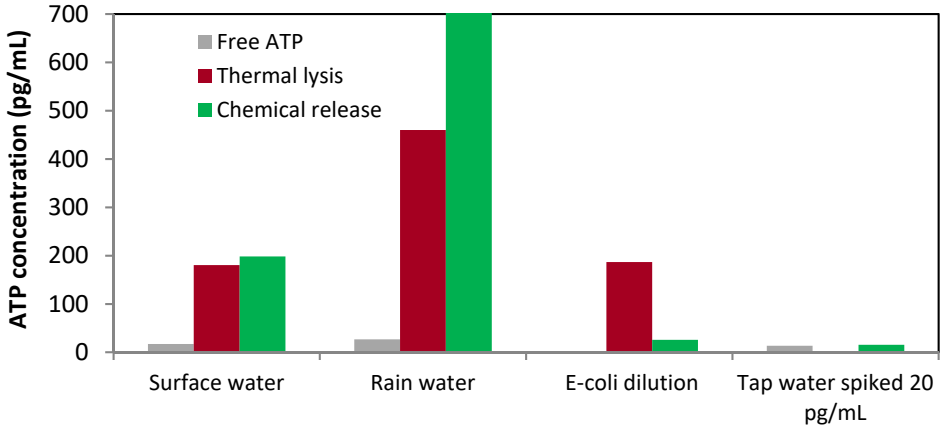

Figure 3. ATP released from the cells by chemical and thermal methods in different types of water. Free ATP is the ATP measured without any releasing step.