



Info-package 4

Water Engineering Companies

Fact Sheet 4.2 – Sensors and other innovative tools for monitoring reclaimed water quality: facts and figures



SUWANU EUROPE is a H2020 project aiming to promote the effective exchange of knowledge, experience and skills among practitioners and relevant actors on the use of reclaimed water in agriculture. This document is part of 5 factsheets in Info-package 4 addressed to water engineering companies, that describe different innovations to detect pathogens or the dissolved organic carbon. For the pathogen measurement, the flow cytometry will be used and for the dissolved organic carbon, an UV LED fluorescence sensor will be used. Finally, the upcoming technology is presented.

INTRODUCTION

1. Flow Cytometry

The majority of waterborne bacteria are non-cultivable and do not form colonies on available standard microbiological culture media. As a consequence, these bacteria are not picked up by cultivation-based detection methods. Among the cultivation-independent methods, flow cytometry is prominent due to its speed and reproducibility of results with different types of water. The determination of the bacterial concentrations can be performed within 15 minutes and is possible in an online format. Apart from pure quantification the technology allows the differentiation between intact (living bacteria) and membrane-compromised bacteria (dead/damaged).

2. UV Led Sensor

Continuous online monitoring of dissolved organic matter (DOM) is urgent for the future smart cost-effective control during water treatment. On the other hand, frequent monitoring for dissolved organic carbon (DOC) and toxic disinfection byproducts (DBP) is relatively expensive and time-consuming. As a result, many agencies are highlighting the need for surrogate monitoring of DOC and estimation of DBPs formation potential. Hence it is necessary to develop a kind of cheap, small, energy-efficient but sensitive sensor, which can provide real-time feedback signals for automatic optimization of operation parameters and estimation of DBPs formation during water treatment. Spectral measurements including UV absorbance and fluorescence signals that are associated with bulk DOM, offer particularly promising solutions for frequent online monitoring.

3. Upcoming innovation

Despite all efforts to develop real-time monitoring systems, there is still a lack of robust, continuous, accurate and verifiable real time devices that demonstrate potential for large-scale implementation. Their widespread application has been limited due to an inability to reliably obtain accurate, cost-effective water quality data. On the other hand, the majority of the developed online monitoring systems are direct adaptations of traditional, laboratory-based analytical methods, not originally designed for field applications. Additionally, they are required to operate in extreme and variable environments whilst still obtaining accurate and reproducible results. Consequently, these methods require frequent calibration, maintenance and often consume large quantities of chemical reagents. In addition, the analysers often suffer from cross responses due to matrix variations between the standards and samples analysed, as the measurement conditions are not controlled. There are also significant economic and logistics costs associated with maintenance of remote equipment due to the difficulty to detect problems such as sensor fouling.

1. FLOW CYTOMETRY

1.1. Technology

The membrane integrity of a cell is a well-accepted criterion for characterizing viable (active or inactive) cells and distinguishing them from damaged and membrane-compromised cells. This information is of major importance in studies of the function of microbial assemblages in natural environments, in order to assign bulk activities measured by various methods to the very active cells that are effectively responsible for the observations. The principle of this approach is to use simultaneously a permeant (SYBR Green; Molecular Probes) and an impermeant (propidium iodide) probe and to take advantage of the energy transfer which occurs between them when both probes are staining nucleic acids. A full quenching of the permeant probe fluorescence by the impermeant probe will point to cells with a compromised membrane, a partial quenching will indicate cells with a slightly damaged membrane, and a lack of quenching will characterize intact membrane cells identified as viable.

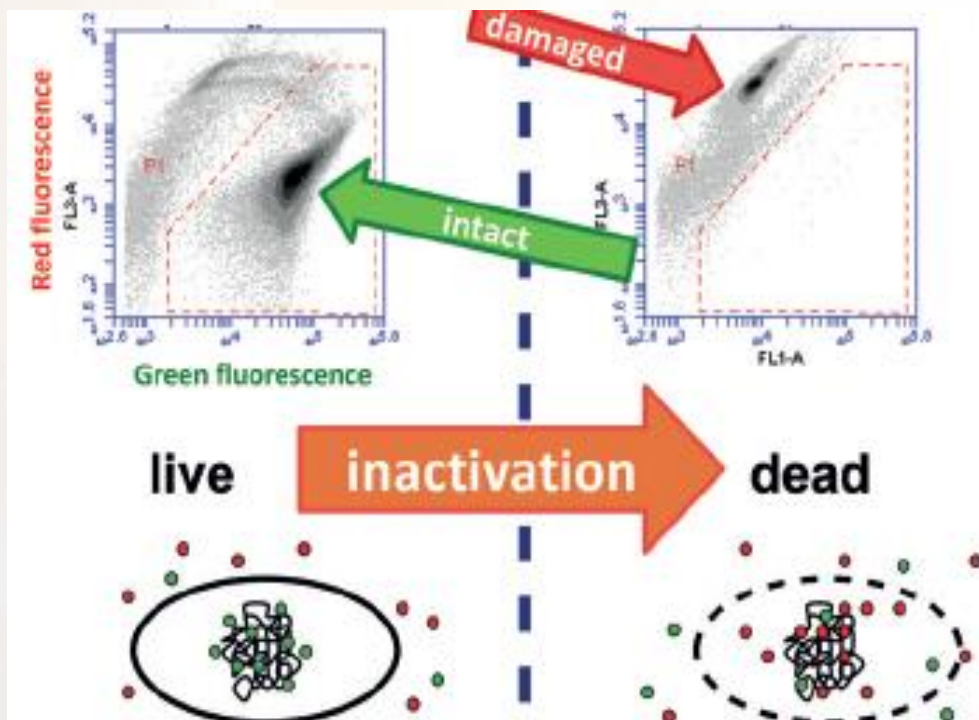


Figure 1: Exemplary flow cytometry density diagram of a bacterial suspension with living/ intact membrane cells (in red-dotted area), resp. with dead damaged membrane cells (outside of the dotted area) after colouration with two dyes

1.2. Application in Water Reclamation

As for other water types, flow cytometry offers fast and reliable determination of bacterial cell numbers in the field of monitoring water reclamation processes. Based on the fact that the detection is not based on the cultivation of the bacteria, the entire bacterial population in the water is measured independent of their growth requirements. While traditional hygienic indicator bacteria such as coliforms, intestinal enterococci or *Clostridium perfringens* are typically not detectable after membrane filtration and total colony counts are only available after 2–3 days, flow cytometry offers a sound database for the microbiological assessment of the efficiency of different water treatment steps. The method is compatible with the 'Hazard analysis and critical control points' (HACCP) concept as the rapid detection of changes in the microbiology provides a good basis for process control decisions.

2. LED UV FLUORESCENCE SENSOR

2.1. Technology

The LED sensor, which measures the UV280 absorbance, protein-like and humic-like fluorescence simultaneously, is feasible to monitor chromophores and fluorophores with good sensitivity and accuracy. The liquid chromatography with organic carbon detector combined with 2D synchronous correlation analysis further demonstrated how the DOM components of large molecular weight were transformed into small moieties as a function of the decrease of humic-like fluorescence. High performance size exclusion chromatography with multi-UV absorbance and multi-emission fluorescence scans are applied to spectrally characterize samples.

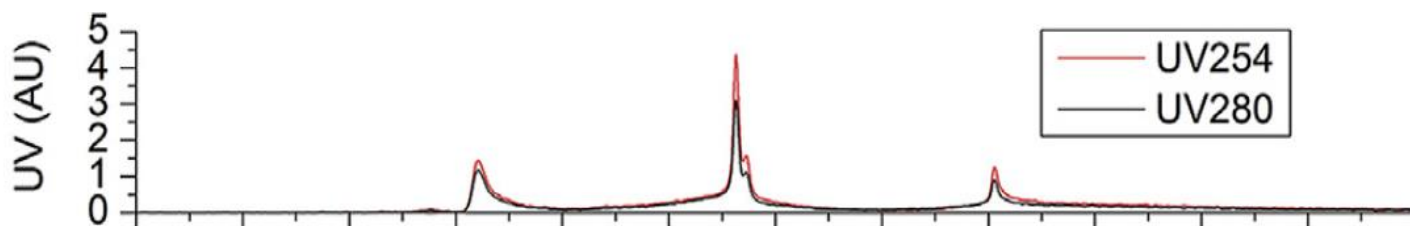


Figure 2: Picture of a spectrophotometer with a diagram showing the peaks measured at UV254 and UV280

2.2. Application in Water Reclamation

Chlorination is the most widely used disinfection practice during water treatment, because it is effective against most pathogens and provides residual disinfecting capacity. However, toxic disinfection byproducts (DBPs) unavoidably form due to reactions between chlorine and natural organic matter (NOM), which has caused serious public health concerns. Many studies suggest that DBPs are cytotoxic and genotoxic and chronic exposure to DBPs is associated with bladder and colon cancer in humans. Being able to detect this products with LED UV fluorescence sensor, increases our understanding and control, which could reduce the concentration of these products during the treatment.

3. UPCOMING INNOVATION

3.1. Technology

For the particular case of pathogen detection in water there is a promising perspective for photonic sensors that improve biological detection taking advantage of the natural fluorescence property of some bacteria, such as *Escherichia coli*. The pathogen sensor proposed by AIMEN aims to detect natural bacteria fluorescence in real water sample in situ measurements. A thorough study with real water samples will make the difference with the existing state of the art in this field. This sensor will not give an accurate *E. coli* quantification, but will perform a monitoring that can establish an estimation of pathogen presence. This has to be understood as an early alarm system, that would imply the need of further lab analysis to confirm the presence and CFU of pathogens in the water. It is also not very specific, but it requires no water treatment, no reagents, functionalization or sample pre treatment, which makes it cheaper and easy to handle.

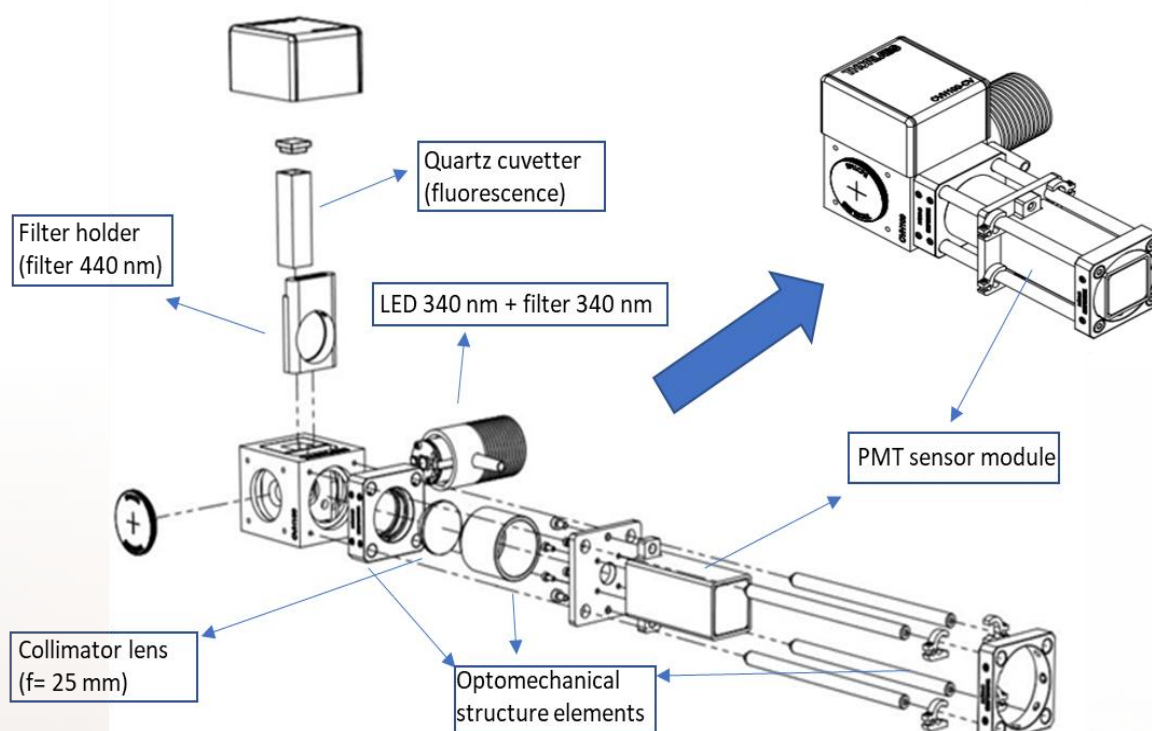


Figure 3: PMT sensor setup suggested by AIMEN for fluorescence measurements of *E. Coli*

3.2. Application in Water Reclamation

There have been some sensors developed to measure natural fluorescence of these bacteria in real conditions without sample treatment and using water circulation systems to reproduce an in-line water monitoring solution. However, there is no report of any of these sensors being used in real sites or monitoring water real samples from water treatment plants. But with the pathogen sensor proposed by AIMEN, this sensor would be an in-line real time monitoring arrangement, continuously measuring fluorescence emission in a bypass in the water treatment plant. If the sensor works properly, it will be a breakthrough in monitoring real water samples outside laboratory conditions without sample treatment, reagent addition or expensive devices.

5. References

- Dartnell, L. (2013). Fluorescence characterization of clinically-important bacteria. PLoS one vol. 8,9.
- Edberg, S. (2000). Escherichia coli: the best biological drinking water indicator for public health protection. J. Appl. Microbiol., 88, 106S-116S.
- Hesari, N. (2016). A biosensor platform for rapid detection of E. coli in drinking water. Enzyme and Microbial Technology vol. 83, Pages 22-28.
- Hongying, Z. (2012). Quantum dot enabled detection of Escherichia coli using a cell-phone. The Analyst vol. 137.
- Kilungo, A. (2013). Continuous Real-time Detection of Microbial Contamination in Water using Intrinsic Fluorescence. doi: 10.4172/2155-6210.S12-002. J Biosens Bioelectron S12:002.
- Li, W. T., Jin, J., Li, Q., Wu, C. F., Lu, H., Zhou, Q., & Li, A. M. (2016). Developing LED UV fluorescence sensors for online monitoring DOM and predicting DBPs formation potential during water treatment. Water research, 93, 1-9.
- Simões, J. (2018). Continuous and Real-Time Detection of Drinking-Water Pathogens with a Low-Cost Fluorescent Optofluidic Sensor. Sensors (Basel, Switzerland) vol. 18,7 2210.
- Wildeboer, D. (2010). Rapid detection of Escherichia coli in water using a hand-held fluorescence detector. Water Research vol. 44, Pages 2621-2628.
- Zulkifli, N. (2018). Detection of contaminants in water supply State-of-the-art monitoring technologies and their applications. Sensors and Actuators B 255 (2018), 2657–2689.



CONTACTS:

Coordinator

Rafael Casielles (BIOAZUL SL)
 Avenida Manuel Agustin Heredia nº18 1º4 Málaga (SPAIN)
 Mail | info@suwanu-europe.eu Website | www.suwanu-europe.eu

CONTACTS:

Responsible for Factsheet

Andres Acosta (TTZ Bremerhaven)
 Am Ludeneich 12 27572 Bremerhaven (GERMANY) | Website |
<https://www.ttz-bremerhaven.de/de/>



THIS PROJECT HAS RECEIVED FUNDING FROM
 THE EUROPEAN UNION' HORIZON 2020 RESEARCH
 AND INNOVATION PROGRAMME
 UNDER GRANT AGREEMENT N. 818088

