Ultrasound Manipulation of Bacteria in Drinking Water for Attenuated Total Reflection Infrared (ATR-IR) Spectroscopy

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Introduction

Freshwater is a finite resource, essential for agriculture, industry and even human existence [1]. Different types of pathogenic bacteria can be present in water sources. For example, *Escherichia coli* (*E.coli*) is an indicator for fecal contamination of drinking water [2]. Therefore, fast, effective and sensitive monitoring of microbiological pathogens is needed to evaluate the presence of waterborne diseases or contamination.
In the food and pharmaceutical industries, infrared (IR) spectroscopy is applied for analysis of microorganisms for rapid identification and screening of life-threatening pathogens, biotechnological process control and microbiological quality control [3]. It provides qualitative and quantitative information in a non-destructive and label-free manner, because inherent vibrations of molecular vibrations are detected [4]. For measurements in water, which is a strong absorber in the mid-IR region and thus allows only interaction lengths in the range of a few micrometers, attenuated total reflection (ATR) configurations are the most commonly employed. Using this technique, the light is totally reflected within the optically denser ATR element, forming an evanescent field that interacts with the sample at typical penetration depths of approximately 1 to 2 µm. To improve sensitivity of ATR-IR experiments, bacteria could be enriched in the evanescent field for example through sedimentation [5]. As an alternative, ultrasound (US) particle manipulation as a mighty tool for separating suspended particles from their host liquid [6] has shown potential in biomedical analytic tasks like cell separation [7], bead-based bioassays [8] and concentration of bacteria in water [9]. Moreover, the formation of conglomerates, due to ultrasonic excitation of yeast suspension enabled US enhanced IR spectroscopy for in-line monitoring of yeast in a bioreactor [10,11].
Our current work aims at developing a device based on ultrasonic particle manipulation and ATR-IR spectroscopy for measuring bacteria in drinking water.

Experimental

We developed an acoustofluidic cell made out of aluminum, equipped with an ultrasound composite transducer consisting of a 10 mm PZT disc (lead zirconium titante, type PIC 181, PI Ceramics, Lederhose, Germany) with wraparound silver electrodes glued to a Macor cylinder with a two-component epoxy resin (Polytec EP 630, Polytec PT GmbH, Karlsbad, Germany). This cell was mounted on a custom-built ATR fixture (US-ATR-IR Sensor; cf.: Figure 1) and embedded in a Fourier-transform (FT)-IR spectrometer (Bruker, Tensor 27, Ettlingen, Germany). ZnS was chosen as material for the ATR crystal due to its refraction index of 2.35 and VIS transparency, which enables observation of the US experiments by a conventional camera. The US transducer was operated at a frequency of approx. 2.6 MHz.

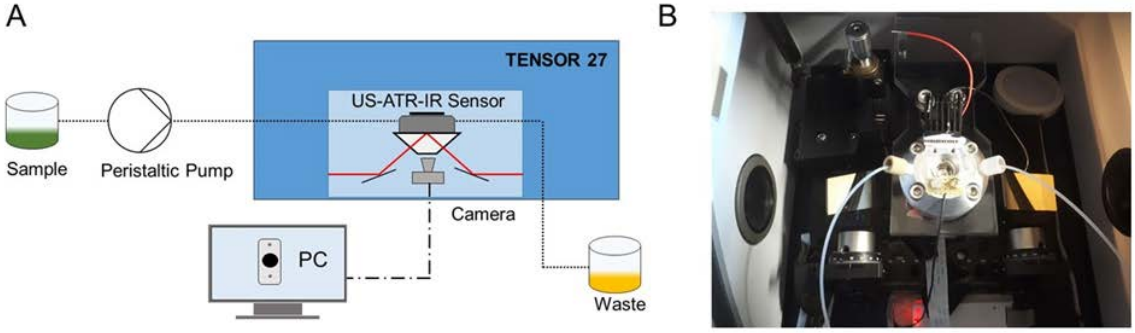

Figure 1: (A) Schematic of the experimental setup. (B) Picture of the custom-made ATR setup incorporated in the sample compartment of the Tensor 27 FT-IR spectrometer.

Results

The acoustofluidic cell of the US-ATR-IR sensor was filled with a 5 mg/ml *E.coli* suspension using a peristaltic pump. When US was turned on, the formation of *E.coli* conglomerates could be observed through the ATR element via a camera (cf.: Figure 2A,B). After 15 min of US exposure, a spectrum was recorded featuring no absorption bands (Figure 2C, black solid line) of *E.coli*, indicating that bacteria agglomeration takes place above the sensitive region of the ATR element. Turning the US off followed by 12 minutes of sedimentation time, an IR spectrum was recorded featuring the typical absorption bands of bacteria, indicating the presence of *E.coli* in the evanescent field (Figure 2C, red solid line). Subsequently, the US transducer was reactivated and an IR spectrum was recorded, which did not show typical IR bands of bacteria (Figure 2C, green dashed-dotted line). After turning off the US transducer again and 12 minutes of sedimentation time, the recorded IR spectrum depicts characteristic IR features of bacteria again (Figure 2C, blue dashed-dotted line). The recorded spectra indicate that our US-ATR-IR sensor is capable of manipulating *E.coli* suspensions via US.

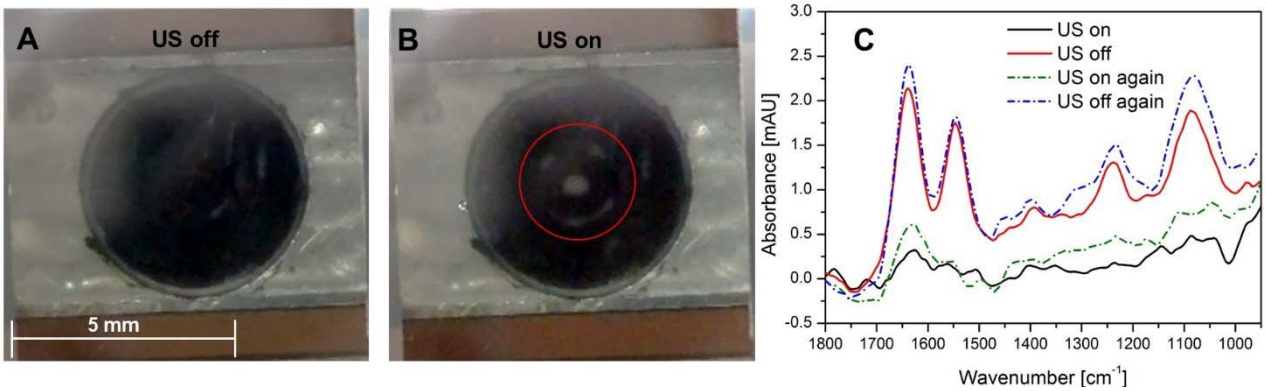

Figure 2: (A) Acoustofluidic cell filled with *E.coli* suspension when US is turned off. (B) Acoustofluidic cell filled with *E.coli* suspension when US is turned on, *E.coli* conglomerates are encircled in red. (C) IR spectra of *E.coli*, black line US turned on, red line US turned off, green dashed-dotted line US turned on again, blue dashed-dotted line US off again.

Conclusion

In this preliminary work, we present our efforts towards developing an US-ATR-IR sensor for monitoring of bacteria in drinking water. We demonstrated that our system is now capable of trapping *E.coli*. By turning off the US, we showed that the bacteria subsequently settled into the evanescent field of the ATR element making them feasible for IR measurements. Furthermore, we are also capable of lifting the bacteria back into the cavity between transducer and the ATR element to clean the sensitive region.
Our next steps will focus on the miniaturization of our acoustofluidic cell and actively push the bacteria conglomerates into the evanescent field.

Acknowledgement

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