Ultrasound enhanced mid-IR spectroscopy for sensing bacteria in aqueous solutions

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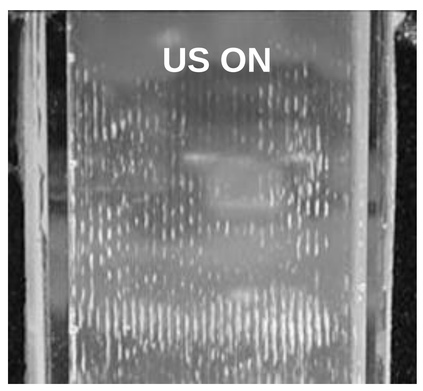
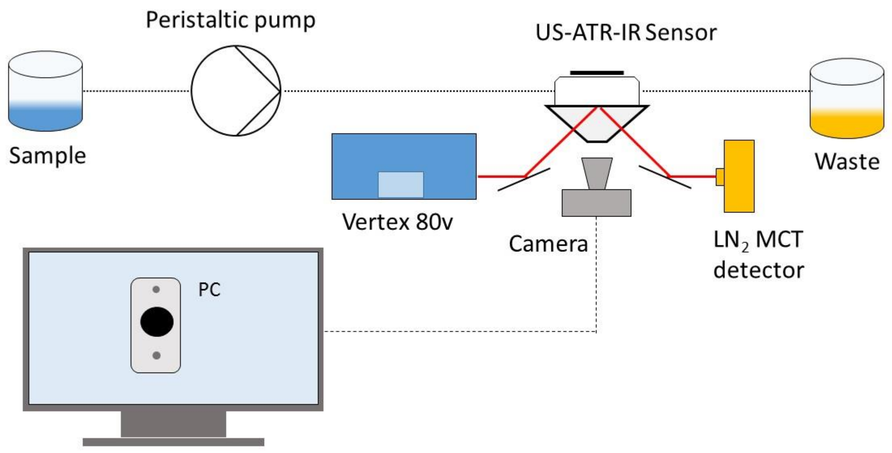
Introduction

In this work, we demonstrate our activities towards the combination of an ultrasound (US) enhanced ATR-IR setup for analysis of bacteria in water. For samples containing low bacteria levels, US-enhanced ATR-IR spectroscopy poses an interesting approach, because the standing ultrasonic wave (USW) can be used in a first step for accumulation of the bacteria in the sample cell. Subsequently, the enriched bacteria solution is forced towards the ATR element for interaction with the evanescent wave.

In order to move biological microscopic particles into the evanescent field of an ATR probe ultrasound has been successfully employed. The potential of such an US-ATR-IR setup has been shown before [1,2].

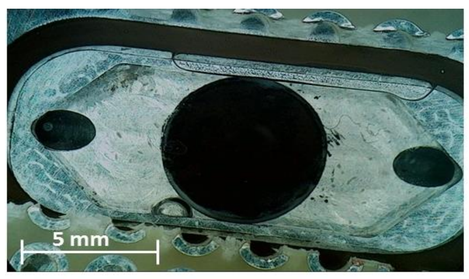


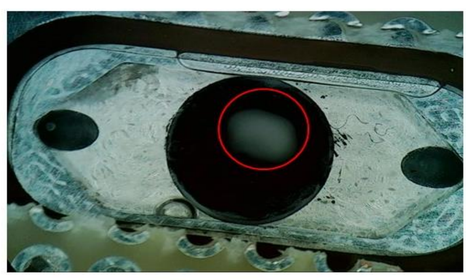
Experimental setup



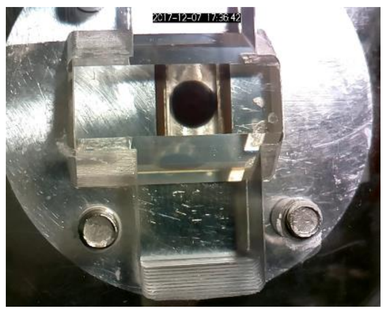
- ATR measurements were performed by guiding the IR beam of a high-end FTIR spectrometer (Vertex 80v, Bruker Optics) through an external custom-made ATR acoustofluidic setup.
- A camera system was implemented for real-time observation of US experiments.

ATR acoustofluidic setup





- Pictures of yeast solution passing through the acoustofluidic setup were recorded by sealing the sample compartment with a glass coverslide. The cell was observed via a reflecting light microscope (Stemi 2000-C, Zeiss). Formation of particle conglomerates (red circle) were observed during the exposure of the solution to US.
- A VIS-transparent ATR crystal made of zinc sulfide was employed in the spectroscopic setup. Therefore real-time monitoring by a camera of the formation of bacterial conglomerates above the ATR crystal is possible.



Ultrasound theory

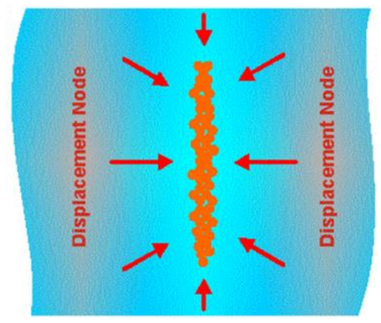
$$F_z^{rad} = 4\pi \cdot \phi(\tilde{\kappa}, \tilde{\rho}) \cdot (ka)^3 \cdot E_{ac} \cdot \sin(2kz)$$

primary radiation force: F_z^{rad}

acoustic energy: $E_{ac} = \frac{p_a^2}{4\rho_0 c_0^2}$

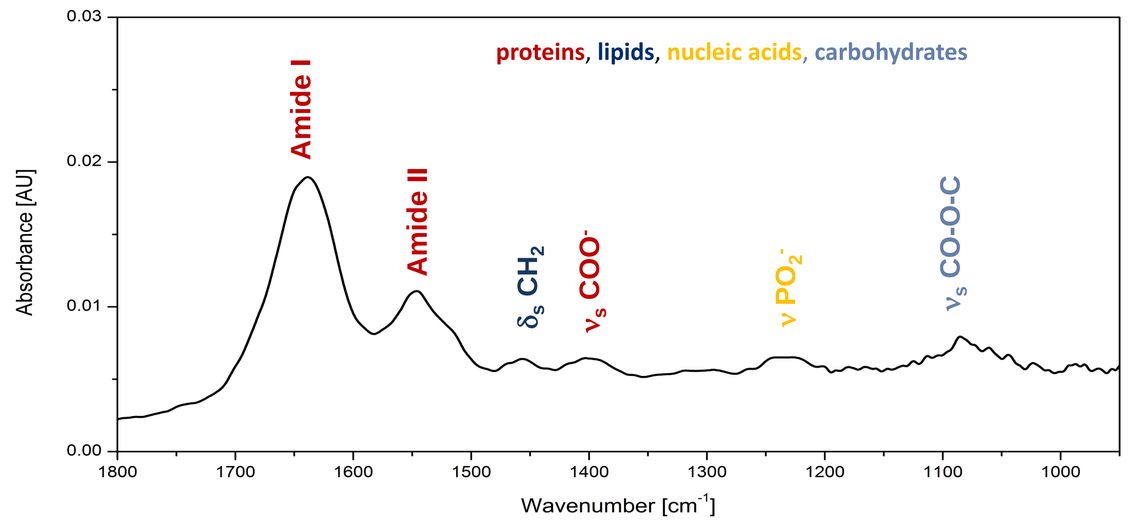
acoustic contrast: $\phi(\tilde{\kappa}, \tilde{\rho}) = \frac{1}{3}\left(\frac{5\tilde{\rho} - 2}{2\tilde{\rho} + 1} - \tilde{\kappa}\right)$

particle size: a; wavenumber: k



- Particles (bacteria) in solution exposed to US are forced into pressure nodes forming conglomerates.
- Through alteration of the US frequency, this conglomerates can be moved towards the ATR crystal into the evanescent field.

ATR-IR spectroscopy



- With the presented custom-made US-ATR-IR setup, a spectrum of *Escherichia coli* was recorded after a settling time of one hour.
- The spectrum shows the typical mid-IR absorption features of bacteria [3].
- Enrichment of bacteria was possible above the ATR crystal but IR spectra of bacteria conglomerates were not recorded yet.

Conclusions & Outlook

- An US-ATR-IR setup was successfully assembled and showed the potential to be a mighty tool for monitoring the bacterial load of aqueous solutions.
- The retention of yeast suspended in water passing through the acoustofluidic setup underlines the capabilities of US as a concentration step prior to IR experiments.
- In the future, our work will focus on altering the design of the acoustofluidics ATR configuration towards miniaturization as well as to use Vernier quantum cascade lasers as IR radiation source.

Financial support was provided by European Union's Horizon 2020 research and innovation programme within the research project "Waterspy" under the grant agreement no.: 731778. The project is an initiative of the Photonics Public Private Partnership.

[1] Koch, C., Brandstetter, M., Lendl, B. and Radel, S.: Ultrasonic Manipulation of Yeast Cells in Suspension for Absorption Spectroscopy with an Immersible Mid-Infrared Fiberoptic Probe. *Ultrasound Med. Biol.* **2013** 39(6), 1094-1101.

[2] Koch, C., Brandstetter, M., Wechselberger, P., Lorantfy, B., Plata, M. R., Radel, S., Herwig, C. and Lendl, B.: Ultrasound-Enhanced Attenuated Total Reflection Mid-infrared Spectroscopy In-Line Probe: Acquisition of Cell Spectra in a Bioreactor. *Anal. Chem.* **2015** 87(4), 2314-2320.

[3] Baker, M. J., et al.: Using Fourier transform IR spectroscopy to analyze biological materials. *Nat. Protoc.* **2014**, 9, 1771-1791.