USW manipulating the location of suspended yeast cells in close proximity to an in-line infrared spectroscopy probe

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The growing use of biotechnology as a manufacturing route for e.g. antibiotics and other medical compounds stimulates the development of reliable sensors for bioprocess purposes. Vibrational spectroscopy is an optical measurement technique increasingly popular in process analytical chemistry because of its ability to directly provide molecular specific (bio-)chemical information about a given sample. The ATR (Attenuated Total Reflection) spectroscopy is a widely used method for mid-infrared vibrational spectroscopy especially in connection with highly absorbing samples like e.g. aqueous solutions. Only a thin film of some micrometers in the proximity of the ATR sensitive element is spectroscopically analyzed, beyond this evanescent field the instrument is "blind". This opens the possibility to employ the radiation forces within an USW to manipulate the whereabouts of suspended particles – biological cells in the case of a bioreactor - relative to the ATR. More precisely the field is used to either push the particles towards the optical sensor or away from it. This enables one to detect the infrared spectrum of cells and the supernatant independently.

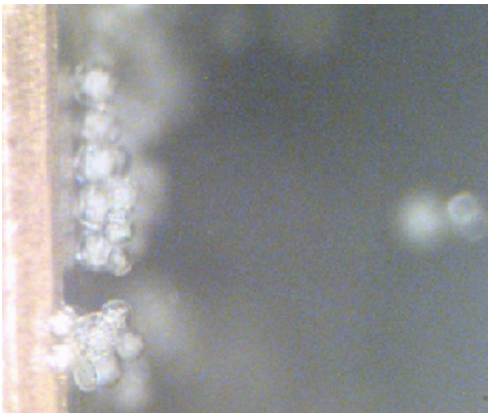

Fig. 1. Resin beads suspended in methanol being pushed to a brass dummy probe acting as a reflector for the USW.

Light micrographs (Fig.1) suggested, that the task was successfully accomplished with polystyrene beads suspended in methanol, aggregates were manipulated to and from the reflector of an ultrasonic resonator. Feasibility was confirmed by infrared absorption spectra recorded when PTFE particles suspended in tetrahydrofuran were manipulated in the evanescent field of a truncated, cone-shaped ATR tip[1].

Two key factors are of great importance when bioprocess monitoring and control is in the focus of attention. Firstly, the time resolution of a measurement system has to be sufficiently higher than the

[1] Radel et al. *Observation of particles manipulated by ultrasound in close proximity to a cone-shaped infrared spectroscopy probe.* Ultrasonics (2009), in press, doi:10.1016/j.ultras.2009.09.030

generation time of the observed microorganism. Secondly, the delivered information necessarily has to reflect the condition of the suspension within the bioreactor as close as possible.

We have recently reported the successful application of an USW within an on-line ATR flow cell[2]. The USW was employed to increase the settling speed of the cells, an infrared spectrum of the culture could be obtained in approx. 70 seconds, this meant an acceleration by a factor of 2.5. Infrared spectra in this set-up were taken on-line in bypass to the bioreactor, resulting in a possibility of a slight temperature shift and the risk of contamination.

In the present work we set out to develop a robust, fast *in-line* sensor delivering chemical information of the particles and the liquid of a suspension combining the two technologies.

The device was constructed to be inserted into a standard port of a bioreactor, hence the environment during measurement shall be guaranteed to be unaltered. Moreover, due to the high cell concentration that can be established in seconds in the evanescent field of the ATR by the USW, fast response times were warranted. A novel industrial fibre-optic probe with a flat ATR element was used, as this element constituted the reflector of the ultrasonic resonator (see Fig.2).

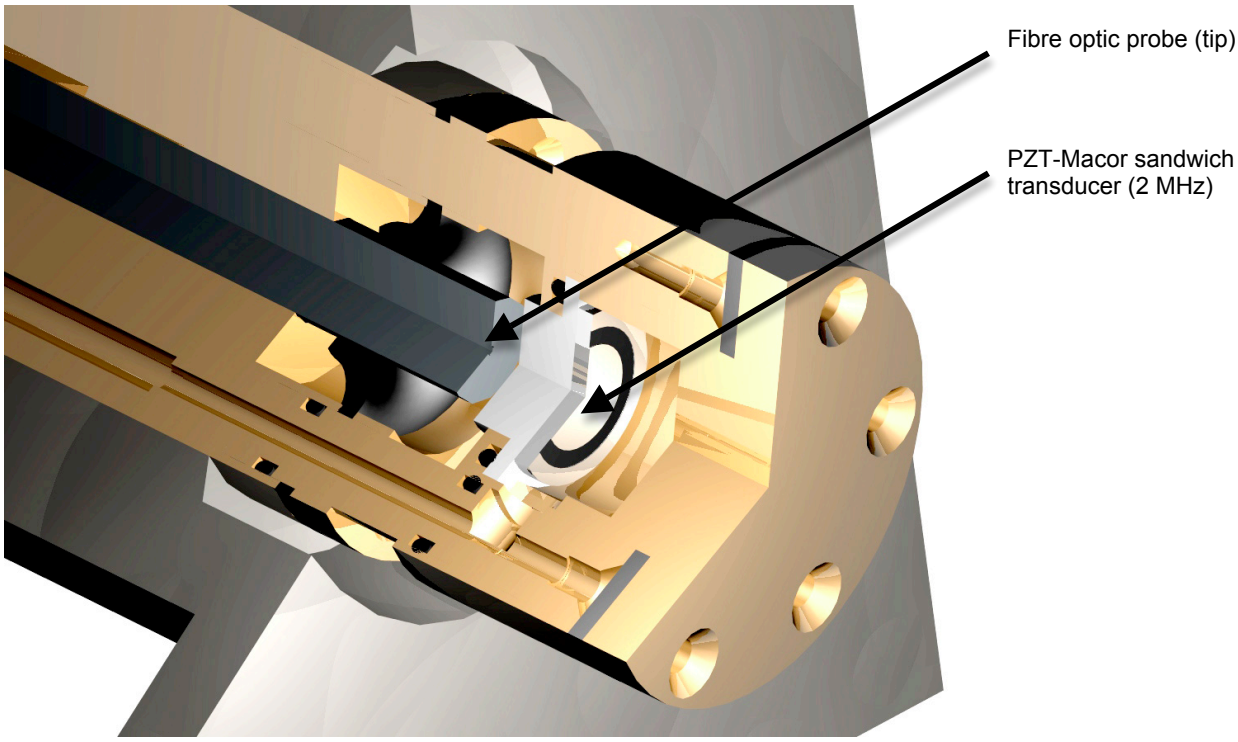

Fig. 2. Tip of the prototype of an in-line ATR probe exploiting an USW to actively control the spatial residue of biological cells in suspension.

Infrared spectra showing an USW populating and depopulating the sensitive zone of the ATR will be presented. It was possible to assess a yeast culture independently from its supernatant. Measurements showed lower absorption when compared to results of wet sediments (which cannot be produced within a bioreactor). Infrared spectra of the culture in suspension were measured within some ten seconds, no contamination of the ATR was observed.

[2] Radel et al. *Ultrasonic particle manipulation exploited in on-line infrared spectroscopy of (cell) suspensions.* Elektrotech. Inftech. (2008) vol. 125 (3) pp. 76-81, doi:10.1007/s00502-008-0514-3