Ultrasound in Med. & Biol., Vol. 39, No. 6, pp. 1094–1101, 2013

0301-5629/$ - see front matter

http://dx.doi.org/10.1016/j.ultrasmedbio.2013.01.003

● *Original Contribution*

ULTRASONIC MANIPULATION OF YEAST CELLS IN SUSPENSION FOR ABSORPTION SPECTROSCOPY WITH AN IMMERSIBLE MID-INFRARED FIBEROPTIC PROBE

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(*Received* 26 *September* 2012; *revised* 8 *January* 2013; *in final form* 10 *January* 2013)

Abstract—Recent advances in combining ultrasonic particle manipulation with attenuated total reflection infrared spectroscopy of yeast suspensions are presented. Infrared spectroscopy provides highly specific molecular information about the sample. It has not been applicable to in-line monitoring of cells during fermentation, however, because positioning cells in the micron-thin measurement region of the attenuated total reflection probe was not possible. Ultrasonic radiation forces exerted on suspended particles by an ultrasonic standing wave can result in the buildup of agglomerates in the nodal planes, hence enabling the manipulation of suspended cells on the microscopic scale. When a chamber setup and a prototype in-line applicable probe were used, successful control over the position of the yeast cells relative to the attenuated total reflection sensor surface could be proven. Both rate of increase and maximum mid-infrared absorption of yeast-specific bands during application of a pushing frequency (chamber setup: 1.863 MHz, in-line probe: 1.990 MHz) were found to correlate with yeast cell concentration. (E-mail: stefan.radel@tuwien.ac.at) © **2013 World Federation for Ultrasound in Medicine & Biology.**

Key Words: **Ultrasonic particle manipulation, Fourier transform infrared spectroscopy, Acoustic radiation force, Ultrasonic standing wave, Attenuated total reflection, *Saccharomyces cerevisiae*.**

INTRODUCTION

When an ultrasonic standing wave (USW) is applied to a suspension, radiation forces are exerted on the suspended particles. The origin of these forces are the spatial gradients of the sound wave's acoustic pressure (King 1934). Depending on the mass density and speed of sound of the particles and the host liquid, respectively, the particles are driven into regions of vanishing displacement or pressure. Therefore, the nodes within a standing wave are regions where particle aggregation (or thinning) can be observed; solid particles typically travel into the pressure nodes of the sound field. Acoustic radiation forces have previously been used for reliable sample concentration in sensor applications (Coakley 1997; Hill et al. 2004), including medical environments (Barnes et al. 1998). Furthermore, Saito et al. (2002) showed that locomotive particles could be manipulated/positioned by a combination of perpendicularly aligned ultrasonic standing wave fields. The ability of an USW to deposit particles on a surface has been investigated with functionalized surfaces (Hawkes et al. 2004) and by optical means (Glynne-Jones et al. 2009).

Mid-infrared (mid-IR) Fourier transform infrared (FT-IR) spectroscopy is a well-developed method in chemical analysis. In combination with attenuated total reflection (ATR) sensing elements, (mid-IR) spectroscopy appears to be a very promising method for process monitoring, particularly useful in (bio)process applications. The ATR technique exploits the occurrence of total reflection of light at the interface of two media with different refractive indices and, thus, only has a detection range of some microns, that is, within the evanescent field. New devices and concepts for advanced chemical analysis based on this technique have been developed over the years (Harrick 1967). For measurements in suspension, additional measures are necessary to bring a sufficient amount of suspended particles into the evanescent field region. Recently, the mid-IR spectroscopic assessment of biological cell sediment onto a horizontal ATR element was reported (Jarute et al. 2004; Schnöller and Lendl 2004). Sedimentation, however, is not an option for in-line measurements, for example, in bioreactors.

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The recording of an FT-IR absorbance spectrum of a given sample consists of two successive measurements. In each measurement the intensity recorded at the detector for different wavenumbers $\tilde{\nu}$ (reciprocal λ, expressed in cm^{-1}) is recorded. First, the background spectrum I_0 (cm^{-1}) is obtained where all components but the sample of interest are present in the optical path of the spectrometer. In the experiments described in this work, this corresponds to the mounted ATR unit with the active part of the ATR element covered with solvent. Subsequently, the sample, here particles contained in the solvent, is placed on the ATR element, and the spectrum I (cm^{-1}) is recorded. Calculation of the absorbance spectrum following the Beer–Lambert law

$$A(\tilde{\nu}) = -\log\frac{I(\tilde{\nu})}{I_0(\tilde{\nu})}$$

yields the specific absorption spectrum of the sample (particles) only, as all other contributions to the achieved light throughput by optical components and solvent cancel out and only the contribution of the particles remains.

An IR absorption spectrum can be acquired in minutes to seconds and delivers specific molecular information about the sample in the optical pathway (Chalmers and Griffiths 2002). In industrial environments it has become state of the art to connect the ATR sensor to the spectrometer with flexible mid-IR conducting fibers. With this technology, the ATR sensor can be immersed in a reactor, thus enabling in-line assessment of the production process.

We set out to exploit the particle manipulation abilities of an USW in ATR spectroscopy. USWs had already been applied with a horizontal ATR unit, attached to a standard FT-IR spectrometer, to improve long-term stability during online bioprocess monitoring (Radel et al. 2010a). In an online configuration, a process stream is continuously taken from the bioreactor and directed to the FT-IR spectrometer. Also, we reported the first successful application of an USW to direct particles of various laboratory suspensions into the evanescent field of an ATR (Lendl et al. 2010).

In continuing these successful attempts to combine an USW and an ATR FT-IR fiberoptic probe, we present our results obtained with prototype setups approaching the research goal of an in-line device, that is, a device in which the ATR sensor is immersed in the bioreactor. With in-line spectroscopy applications in biotechnology in mind, we describe the application on suspensions of *Saccharomyces cerevisiae*, baker's yeast in this work. The choice of yeast as a biological model was guided by availability and simplicity in handling (Hawkes et al. 1997). However, in the context of ultrasonic radiation forces, the spherical shape of the yeast cell is advantageous with respect to the applicability of theoretical results, where spherical particles are usually investigated (Doinikov 1997; Gröschl 1998a; King 1934).

Generally, exposure to an USW does not harm the cells; yeast have been shown to be viable after sonication (Radel et al. 2000b). Moreover, it has been shown that yeast are able to reproduce after ultrasonic arrangement in a cluster (Gherardini et al. 2005). Only when the cells left the pressure nodes of the USW significant alterations in viability were observed (Radel et al. 2000a). Spengler et al. (2000) investigated the effects of primary axial and transverse acoustic radiation forces on yeast in a one-wavelength resonator. They observed a distinct, reproducible pattern of yeast cell clump formation, indicating that the transverse acoustic radiation force (resulting from the energy density distribution over the transducer surface) can have a strong effect on cell distribution. As yeasts are of industrial relevance, reports on the use of ultrasound in respective bioprocesses exist (Chisti 2003; Palme et al. 2010).

To combine the optical and ultrasonic techniques it was necessary to implement an USW in proximity of the ATR probe tip. One way to accomplish this was to use the ATR probe as the reflector of an ultrasonic resonator. Therefore, the USW was built up between an ultrasonic transducer and an ATR fiber probe. As a consequence, suspended cells agglomerated in the pressure nodal planes, parallel to the surface of the ATR probe (Radel et al. 2010b). As the position of the nodal planes depends on the driving signal, it was expected that the precise location of these cell aggregates relative to the evanescent field could be controlled by the ultrasonic frequency. Figure 1 shows this effect on the example of polystyrene beads in tetrahydrofurane (THF). One can clearly see the particles, which agglomerated in planes. The same behavior was expected for yeast cells in water; however, because of the turbid appearance of yeast suspensions, photographic proof was not feasible.

METHODS

Experimental setups

Two different setups were investigated: a brass/glass chamber containing the suspension with the ultrasonic field oriented horizontally and an USW equipped in-line probe immersed in a vessel that could be inclined at any angle relative to gravitational forces.

In both cases the ATR probe was connected to the spectrometer by flexible silver halide fibers, and both probes contained an ATR unit at the top. Diamond was used as the ATR element because of its optical properties (refractive index) and inertness. The optical setups provided a bandwidth from 600 to 1960 cm^{-1}, a range including the "fingerprint region" most important for the analysis of