Ultrasonic Standing Wave Accelerates On-Line Measurement and Prevents Coating of a FTIR ATR Flow Cell

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Abstract

It has been shown elsewhere that infrared (IR) spectroscopy can be successfully employed for the on-line monitoring of bio-processes. A horizontal attenuated total reflection (ATR) unit connected to a portable IR-cube was used here to measure the IR absorption spectra of supernatant and microorganism separately. The common problem of bio-film formation on the ATR was addressed before, e.g. by chemical means. We present a novel method employing the principles of ultrasonic particle manipulation to avoid and potentially remove this coating brought about by the use of fermentation broth.

A novel flow cell for a horizontal ATR was developed that decreases measurement time and the undesired formation of bio-films on the ATR surface. An ultrasonic standing wave (~2MHz) is build up between a horizontal transducer and the ATR crystal. Yeast cells in suspension were agglomerated within certain regions by the ultrasound field and therefore settled about 3-4 times faster on the ATR when the field was switched off compared to the slow sedimentation of freely dispersed cells. After the IR spectrum had been measured, the same sound field was used to actively lift the settled material from the optical sensitive surface which therefore could be rinsed away more effectively.

Keywords

FTIR, actuator, ultrasound, ultrasonic particle manipulation

INTRODUCTION

Due to the direct molecular specific information provided by infrared spectroscopy this technique is able to predict the concentration of several target compounds, with minimal or no sample preparation and reagent consumption [3].

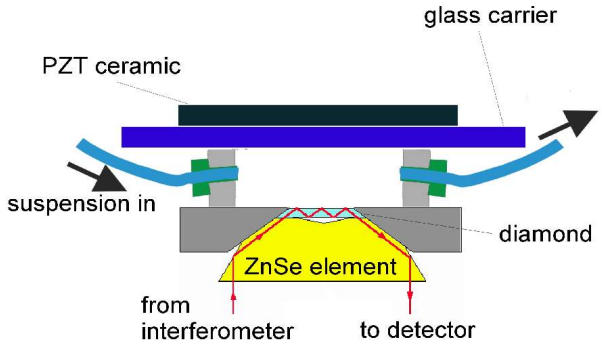

Figure 1. Sketch of flow cell

The high potential of the FTIR sensor employed here with a horizontal attenuated total reflection (ATR) device is in the possibility to measure dissolved reagents and products (e.g. in case of a yeast fermentation: glucose, ethanol, organic acids,…) in the broth and the chemical composition within the microorganism taking part in a bio-process independently and on-line.

However some disadvantages are brought about by this geometry as the sedimented cells (and other material) tend to build up a coating on the sensing surface [2]. This leads to a decreased longterm stability and sensitivity of the sensor. The possibility to remove such a bio-film chemically was reported [6].

The flow cell presented here was designed to address the problem differently. An ultrasonic standing wave was used to actively manipulate the residue of particles within. Within such a standing wave forces are exerted on particles towards so-called pressure nodes, i.e. regions of vanishing sound pressure of the ultrasonic field. This technique is referred to as ultrasonic particle manipulation.

Ultrasonic separation as exploited here has been increasingly used for particle manipulation recently. Moreover its non-destructiveness for biological cells - in contrast to the well known ultrasonic cleaning and disruptor devices utilizing the effect of cavitation at frequencies of a few ten kHz - was shown, In fact microorganism's like yeast cells are very gentle handed by a megahertz standing wave field [4].

Ultrasonically Enhanced Settling (UES) devices are exploited industrially as cell filters in biotechnology [1].

EXPERIMENTAL

Setup

The used flow cell (see Figure 1) comprises of cavity (cylindrical, 4.3 x 5.2 mm) between a horizontal FTIR ATR element at the bottom and an ultrasonic transducer at the top. The measured infrared absorption takes place in close proximity of a small diamond window (d 3mm), placed on top of a ZnSe focussing element [5].

The PZT[1]-glass compound transducer (PZT cylindrical, 1 x 20 mm, Hoechst Tempax glass 2.77 x 25 mm) on top emits an ultrasonic wave of around 2 MHz (Frequency Power Synthesizer FPS 4025, Psi, Austria) into the liquid. This wave is reflected by the optical element and due the

[1] PZT: lead zirconate titanate

superimposition of the incident wave and the reflection an ultrasonic standing wave is build up.

Protocols

To model fermentation broth 7.5 g/L ordinary baker's yeast (Mautner Markhof Austria Hefe AG) was suspended in NaAc/HAc buffer (50mM, pH 5).

The flow cell was connected to a pump controlled by a computer program which as well was timing the IR measurements. The system containing buffer was filled with suspension at 5 ml/min. Throughput was then switched off to let the cells sediment on the ATR. Subsequently the IR spectrum was recorded (average of 10 scans, mirror excitement 40 kHz). Afterwards the system was rinsed with distilled water.

To test the ability of the ultrasound field to lift deposited material the standing wave field was switched on and off for 10 s, respectively during the rinsing.

For the evaluation of ultrasonic enhanced settling the system was sonicated during the inflow of the suspension and the sound field was switched off together with the pump. The determination of sedimentation speed was achived by taking spectra every 5 s and therefore detecting the increase of signal due to settled yeast cells.

IR spectra are given as absorbency unit [AU], the logarithm of the ratio[2] between the absorbencies of the flow cell filled with the suspension and the background, i.e. the filling with distilled water immediately before the measurement, respectively.

As this background includes a bio-film adhered to the ATR these signals have been further investigated to evaluate the coating in comparing them to the spectrum of the carefully cleaned flow cell in the beginning of an experiment, i.e. when no suspension had been in contact with the ATR before.

RESULTS

Ultrasound applied during rinse

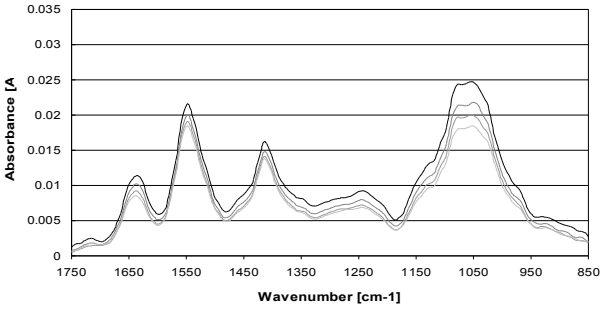

Figure 2. Absorbency spectra of freely settled yeast, Δt~30 min

Figure 2 shows four infrared spectra of the same yeast suspension. A decrease of absorbency and hence resolution was detected compared to the first measurement (black line) for each of the following runs (lines in all figures become lighter as time passed by).

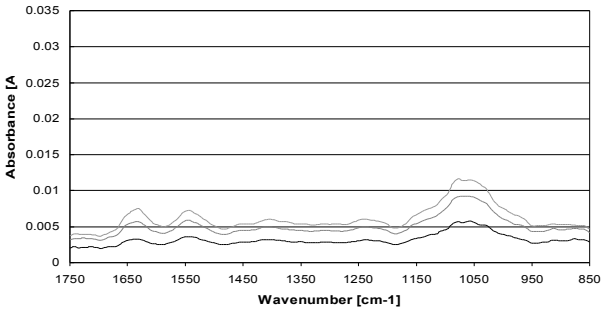

Figure 3. Background spectra of freely settled yeast, Δt~30 min

The respective background, i.e. the increase of absorbencies measured with the flow cell due to a bio-film coating is shown in Figure 3. A significant increase of absorbencies at 1630 cm^{-1} and 1545 cm^{-1} (proteins) and between 1040 cm^{-1} and 1080 cm^{-1} (carbohydrates) for each measurement was detected.

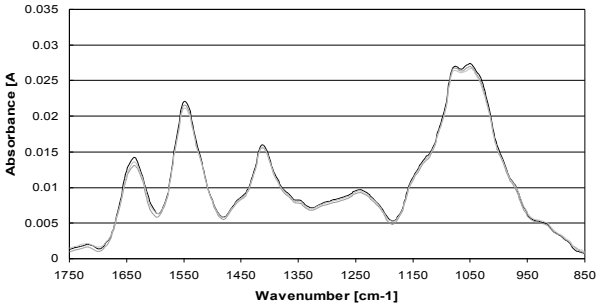

Figure 4. Absorbency spectra of yeast, US protocol, Δt~30 min

The result of the same experiment except that the ultrasonic field was switched on and off during the rinse is shown in Figure 4. A mild decrease of absorbencies was detected. The respective measurement of bio-film in Figure 5 again shows some increase of absorbency due to ATR coating for the protein region but almost no deposition of carbon-hydrates.

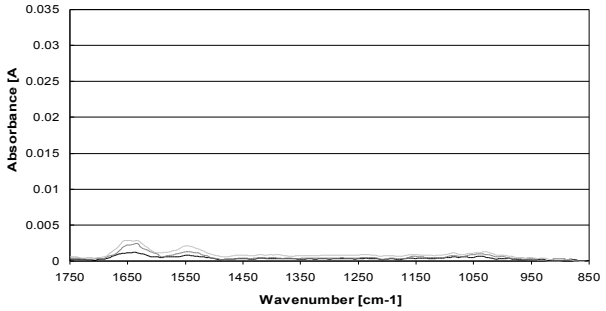

Figure 5. Background spectra of yeast, US protocol, Δt~30 min

[2] Lambert Beer's law: $A[AU]=\log(I/I_0)$.

Ultrasound enhanced settling

When suspension is fed into the flow cell and the flow is switched off, the suspended yeast slowly settle on the ATR surface due to gravity. The process is shown in Figure 6. The rising absorbencies is the result of more and more cells cover the optic. It took ~185 s to reach the maximum absorbency.

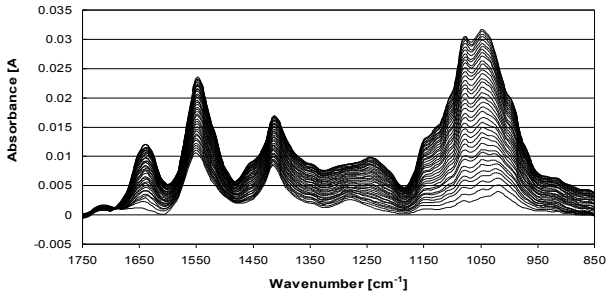

Figure 6. Absorbency spectra of freely settling yeast cells

The increase of settling velocity due to ultrasonic agglomeration of the yeast cells was assessed by having an ultrasonic standing wave present when the flow cell was filled with suspension. The results of subsequent measurements after the sound (and the pump) was switched off are shown in Figure 7. Immediately after the sound stops to prevent the yeast cells to drop a quick increase of absorbencies was observed within 75 s.

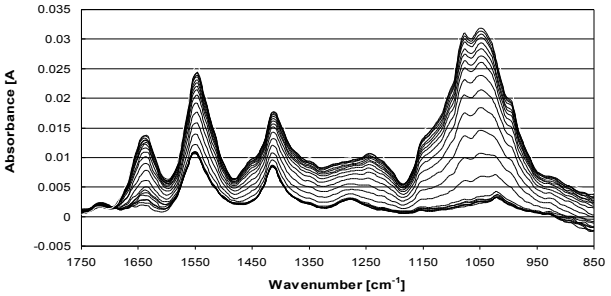

Figure 7. Absorbency spectra during Ultrasound Enhanced Settling

DISCUSSION

The comparison of the measurement results in Figure 2 and Figure 4 delivers a significant improvement in terms of longterm stability when an ultrasonic standing wave was used during the rinse. The decrease of the absorbency signal was much smaller.

Results presented in Figure 3 and Figure 5 might deliver an explanation in the increase of the signal due to bio-film deposition on the ATR was lower when the flow cell was sonicated. Depending on the wavenumber a three to ten times lower absorbencies by the bio-film was detected. Especially the absorbency of carbon-hydrates which are believed to be specifically for the presence of the cells was almost vanishing. Some protein might still be adhered to the ATR detected by the peaks at 1630 cm^{-1} and 1545 cm^{-1}. This very well could be substances in solution or in very small clusters which the ultrasonic standing wave of 2 MHz was not able to lift anymore.

The influence of a standing wave present when the system is filled with suspension was delivered by the settling measurements shown in Figure 6 and Figure 7. Compared to the free settling an equivalent signal intensity was reached in less than half the time (~75 s versus ~185 s) when the ultrasound was applied. The overall appearance as well as the signal strength did not change significantly due to the between the two experiments.

OUTLOOK

The presented results are promising. Above the possibility to remove the sedimented cells from the ATR surface after the measurement it has been shown that it should be possible to avoid the formation of a disturbing bio-film by decreasing the time necessary for a measurement. Hence the combination of the FTIR ATR sensor with the technique of ultrasonic particle manipulation has some potential, however the development of a sensor for the industrial environment more research is necessary.

ACKNOWLEDGEMENTS

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