sonic**catch**

trapping of particles in liquids

by usePAT

#3

Enhanced Process Control for (Bio-)Processes

Ultrasound particle manipulation – a new approach to tackle the challenges in the monitoring of bioprocesses

Process control in biotechnological production mostly relies on readily available parameters such as temperature, pH-value and analysis of specimen taken out of the reactor. Most often, these data points are fed into a model based feedback loop, which can be used steer the process. The main drawback is the use of parameters, which are only indirectly correlated to the performance of the microorganism regarding product yield. A better approach would be the direct measurement of the main constituents of the culture medium which are directly involved in the metabolism of the microorganisms or the measurement of the cells themselves. Spectroscopic instruments based on vibrational spectroscopy (e.g. ATR-FTIR or Raman) are good candidates, since they offer selective chemical information in a non-destructive measurement and can be applied directly into the process in form of in-line probes. Often however, they lack the sensitivity required to determine important components in the liquid phase or are not able to measure the cell composition without sample preparation usually done in offline experiments. Ultrasonic particle manipulation can be used to catch the cells inside the bioreactor and present them to the spectroscopic probe, thus enabling their accurate measurement. In this application note as an example an ethanol fermentation is shown, where the ultrasonic enhanced Raman measurement enabled the in-line determination of the fermentation phase and thus the physiological state of the microorganisms themselves.

Ultrasound particle manipulation

Ultrasound waves are non-audible acoustic waves propagating at frequencies above human hearing and up to several gigahertz. A suitable transducer produces an ultrasonic wave, which is reflected by a second surface, thus forming a quasi-standing wave. The standing wave field exerts forces on particles suspended in the medium and if used correctly, in-situ particle manipulation becomes achievable. With accurate control over the properties of the ultrasonic field, it is possible to precisely steer particle movement and control the position of the particles. sonic**catch** is an add-on to most commercially available in-line probes, which enables the use of ultrasound fields for application in various processes with the goal of enhanced sensitivity, selectivity and stability of the measurement.

In-line Raman spectroscopy

Raman spectroscopy is a valuable tool for direct, fast, non-destructive and label-free investigation of different samples. It offers the possibility of real-time reaction monitoring by analyzing the chemical fingerprint of a specific analyte of interest, hereby making the fast monitoring of several components possible. However, Raman suffers from low signal intensity due to the weakness of the underlying physical effect. In-line probes are currently sufficiently sensitive for the monitoring of the changes of the main composition of the process fluid but fail if critical information of the solid fraction e.g. the microorganism, is sought after. sonic**catch** allows for in-situ catching of particles in the focal point of an in-line probe, acting as an in-situ sample preparation, thus giving new process insight.

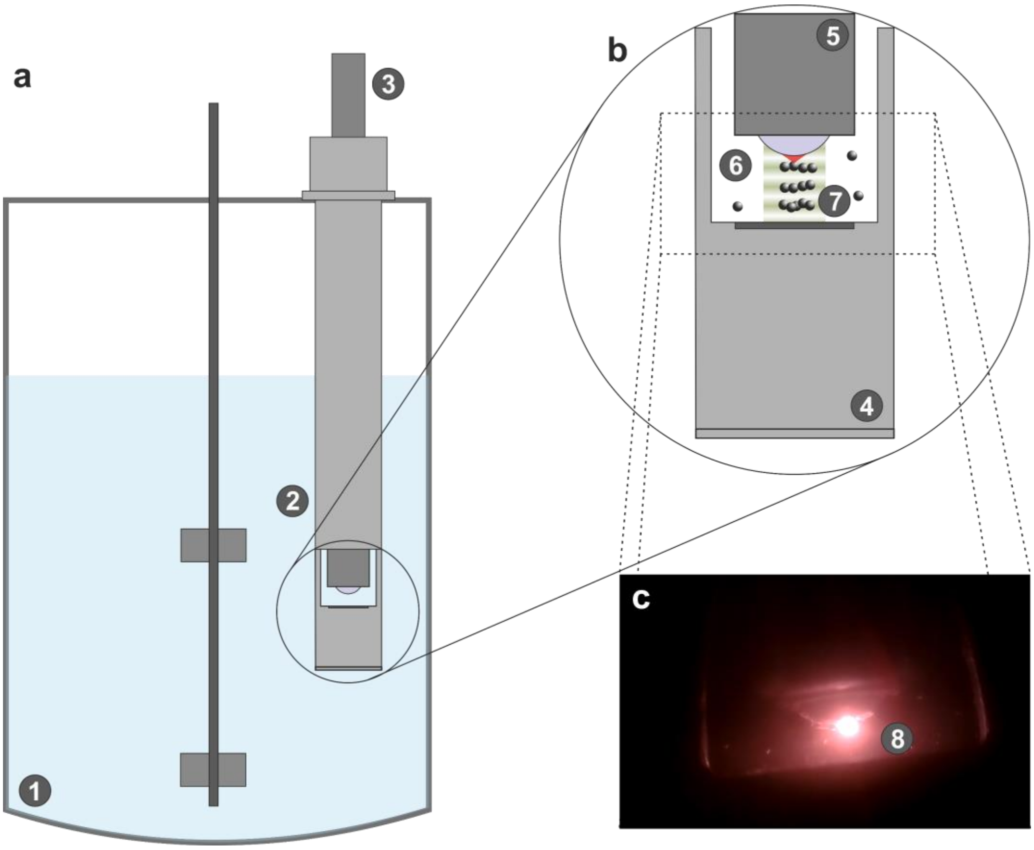

Figure 1: (a) Illustration of a typical bioreactor (1) equipped with an in-line Raman probe (3) inside the sonic**catch** add-on (2). The inset (b) shows the ultrasonic trap in more detail. The in-line Raman probe features a ball-shaped lens (5), which provides a sharp focal area a few hundred µm from its surface. The ultrasonic transducer (7) inside the sonic**catch** add-on (4) produces the ultrasound, which forms an acoustic resonator with the Raman probe (6). Particles, e.g. microorganisms, are trapped in this standing-wave field and are held in place right at the focal spot of the probe, ready to be measured (8). The bright spot in part (c) shows the scattering of the Raman laser on trapped particles.

Ethanol fermentation

To highlight the benefits of the combination of an in-line Raman probe and sonic**catch**, a simple yeast fermentation was conducted. A 5-liter bioreactor was equipped with a standard Raman probe (Process Ballprobe®, 12.7mm diameter, C257 Hastelloy with a sapphire lens, MarqMetrix®), which was fiber-optically coupled to a Raman system (HyperFlux PRO Plus®, 785 nm, 450 mW, 200-3300 cm^{-1}, Tornado Spectral Systems). The probe was fixed into the sonic**catch** add-on (fitted into a standard DN25 ingold port) to form the ultrasonic trap depicted in Figure 1. The integration time of the spectrometer was set to yield a suitable spectrum every 5 minutes. The stirrer speed was set to 600 rpm. sonic**catch** was activated repeatedly during the fermentation, providing clear spectra of either fermentation medium or yeast cells. The alternating selective measurements of both components is displayed in Figure 3d, where the intensity profile for a characteristic band for the medium and the cells is depicted, respectively. The HyperFlux and sonic**catch** were directly coupled to the Siemens PAT infrastructure SIMATIC SIPAT.

Characterization of the medium

With sonic**catch** inactive, distinct spectra of the fermentation broth can be acquired, as shown in Figure 3a. Due to the overlapping spectral features in Raman spectroscopy, chemometric approaches are taken to evaluate the measured spectra. Most commonly Partial Least Squares (PLS) is used to model measured Raman spectra to well-known process parameters. Figure 2a, b and c feature the results of PLS modelling of the recorded medium spectra to the process parameters for this well-known fermentation. The important parameters of glucose, ethanol and biomass concentration can be determined with an error of 0.11 g/l, 0.08 g/l and 0.07 g/l, respectively. When compared to the fermentation process profile, the PLS models show good agreement, as shown in Figure 2d. This means that quantification of several critical process parameters in the fermentation medium is possible with the use of in-line Raman spectroscopy.

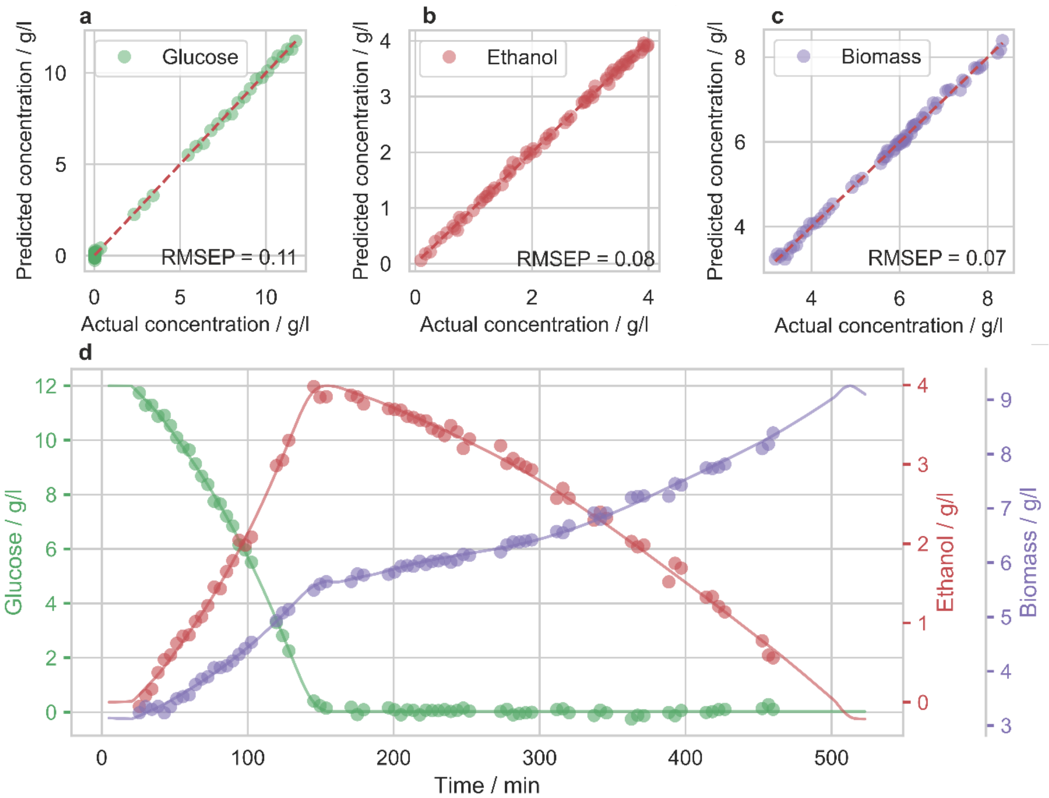

Figure 2: Quantification of the main fermentation components glucose, ethanol and biomass. PLS models were calculated for each parameter and the validation results are displayed in (a), (b) and (c) for glucose, ethanol and biomass, respectively. Part (d) shows the concentration profile of each component over the duration of the fermentation together with modeled data.

Focusing on the cells

When sonic**catch** is activated, cells are collected in the focal area of the Raman probe and representative spectra can be obtained. Figure 3c depicts in-line cell spectra for the whole fermentation. The complex spectral fingerprint can be observed, arising from vibrational transitions from functional groups within the cells, such as proteins, lipids and carbohydrates. These spectra, especially the fingerprint region, hold a great depth of information about the chemical composition of the cells, and with it, about their physiological state.

Again, due to overlapping spectral features, a multivariate data evaluation approach has to be taken. Here, the preprocessed cell spectra are analyzed via Principal Component Analysis (PCA). The scores plot between the first and third principal component shown in Figure 4a reveals a clear distinction between spectra recorded at different phases of the fermentation. Subsequent classification allows for assignment of the spectra to a specific phase of the fermentation, as the cells react to the changes in environment by changing their chemistry, e.g. by starting to accumulate glycogen inside.

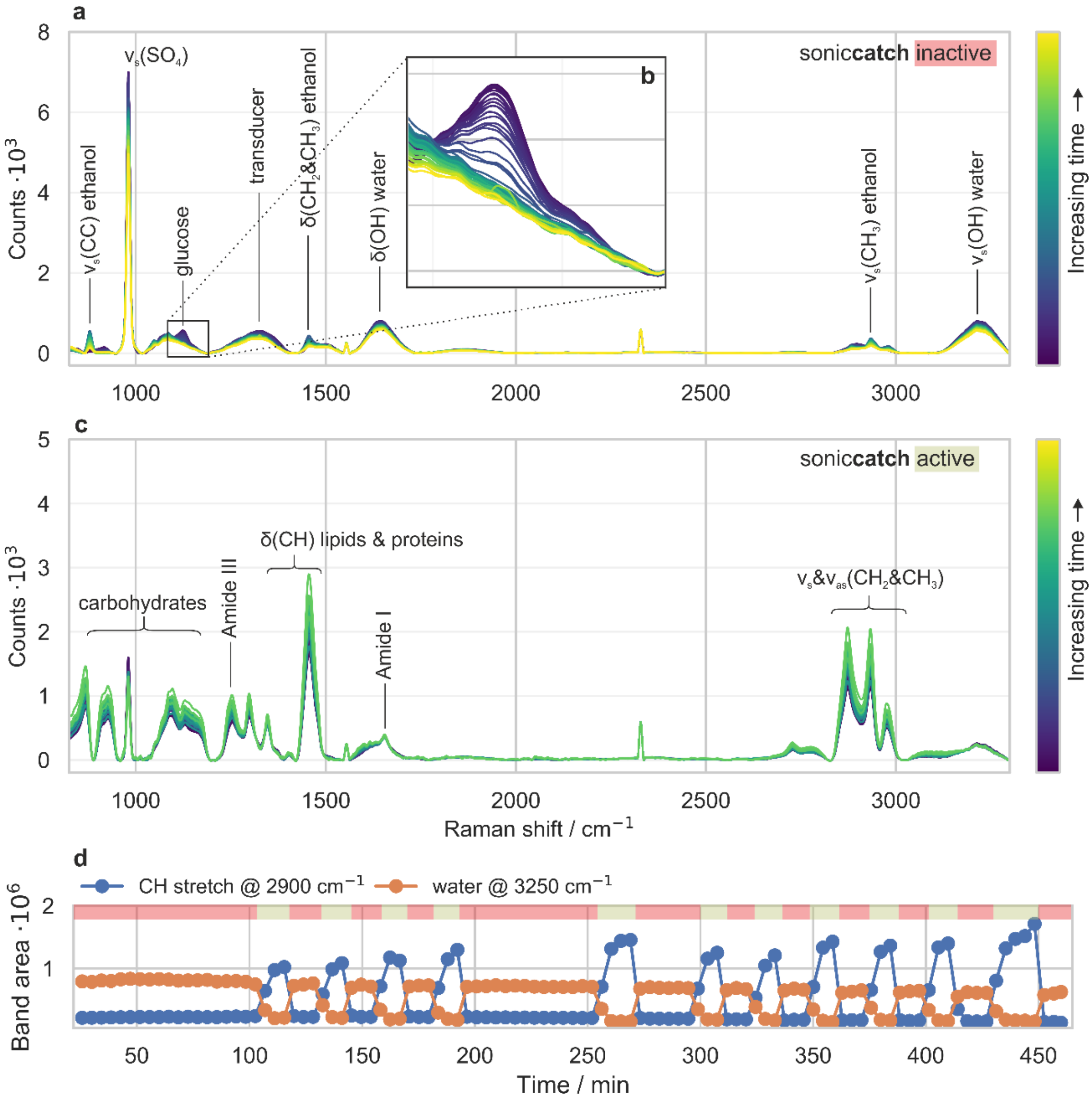

Figure 3: (a) Preprocessed in-line Raman spectra of the fermentation medium (sonic**catch** inactive) over the course of the fermentation. (b) Specific glucose band showing its consumption by the yeast at the beginning of the fermentation. (c) In-situ preprocessed Raman spectra of catched yeast cells (sonic**catch** active). (d) Intensity profile for a characteristic Raman band of organic compounds, as well as for water. The colored regions at the top of part (d) indicate whether sonic**catch** was active or not.

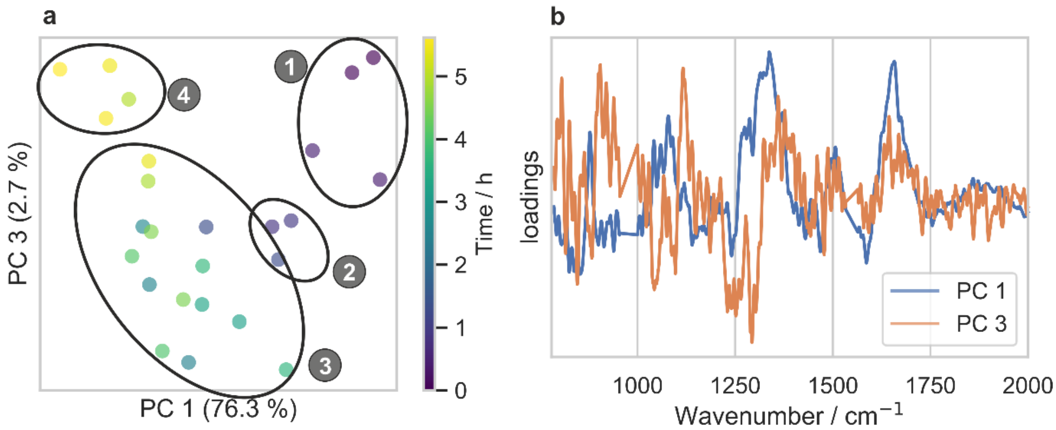

Figure 4: (a) Scores plot for the first and third principal component of the principal component analysis (PCA) on the in-line cell spectra over the fermentation process. (1) In this region spectra in the exponential growth phase of the yeast cells are located. After all glucose has been consumed, the cells prepare to change the metabolism (diauxic shift, (2)). In the respiration phase, the prior produced ethanol is again consumed to further biomass production (3). As all nutrients are consumed, the yeast cells prepare for hibernation (4). (b) The loadings for the PCA for the first and third principal component, showing where in the spectrum the relevant changes are located.

The takeaway

Combining in-line Raman probes with sonic**catch** for the monitoring of fermentation processes allows for the selective measurement of either the fermentation medium or the used microorganisms. The advantages are manifold: the Raman probe itself can be used to quantify multiple components in-line in the fermentation broth, without the need of additional sensors. By catching the cells in front of the Raman probe, a direct measurement of the microorganism used in the fermentation can be facilitated, in-line and without interfering with the process.

In the case presented here, the fermentation phase of the cells can be clearly distinguished. This allows for the direct monitoring of the active fermentation culture and its control without the detour of measuring multiple critical process parameters in their surroundings. This is especially useful, if the process in question uses microorganisms which accumulate the desired product inside their cells. Here, direct measurement of product concentration could be facilitated and would allow for a more efficient overall process performance, or new process insight

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Project: IRAMOUSE, Infrared & Raman Assessment of MicroOrganims by UltraSound Enhancement

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