Introduction

For FTIR spectroscopy of aqueous solutions an ATR (attenuated total reflection) element, in which the infrared light is totally reflected at the interface with the solution, is the instrument of choice. For particle suspensions it is, however, only possible to record spectra of the medium and not the particles.

A method developed and patented by Brandstetter, Radel and Lendl uses the acoustic radiation forces exerted by an ultrasonic standing wave of around 2 MHz to actively position the particles in suspension within and outside the measurement region of the ATR element, respectively.

Results

<table>
<thead>
<tr>
<th>Cell Spectra Acquired Inline</th>
<th>Measurement Mode</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ultrasound frequency 2.410 MHz “pushing frequency”</td>
<td></td>
</tr>
</tbody>
</table>

- Yellow: end of batch fermentation
- Red: onset of Nitrogen limitation $\rightarrow$ trehalose and glycogen start to form
- Black: end of fed-batch phase $\rightarrow$ trehalose and glycogen accumulated

<table>
<thead>
<tr>
<th>Reference Cell Spectra (Offline)</th>
<th>Measurement Mode</th>
</tr>
</thead>
</table>
| Transmission spectra of dried cells on ZnSe slides; | - Frozen
- Sample drawn from bioreactor
- Washed 2x
- Resuspended
- Dried on ZnSe slides |

Trehalose and glycogen formation with onset of Nitrogen limitation can be seen.

<table>
<thead>
<tr>
<th>Medium Spectra Acquired Inline</th>
<th>Measurement Mode</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ultrasound frequency 1.873 MHz “retracting” frequency</td>
<td></td>
</tr>
</tbody>
</table>

- Spectra of the medium during batch fermentation
- Red: glucose is taken up, EtOH is formed
- Blue: metabolism changes to EtOH uptake $\rightarrow$ used up
- During fed-batch fermentation media spectra do not change greatly, since just as much substrate is fed as the cells can take up.

S. Cerevisiae Fermentation

Batch Phase

- Cell biomass ($\rightarrow$)
- $\text{CO}_2$ offgas signal ($\rightarrow$)
- Glucose phase (red): glucose is taken up, cell biomass increases, excess glucose leads to formation of EtOH (red)
- Metabolism changes: EtOH taken up (blue)

Nitrogen Limitation: Fed – Batch Phase

- Substrate limited growth (glucose = growth limiting factor)
- Exponential feed profile $\rightarrow$ exponential growth (biomass)
- Nitrogen limitation:
  - Biomass production inhibited
  - Cells continue to take up glucose and convert it to trehalose and glycogen ("storage carbohydrates")

Ultrasound Enhanced ATR FTIR Probe

- ReactIR 15 with DiComp 1.5 m AgX fibre (Mettler Toledo, USA)
- FDA compliant materials: Macor, Hasteloy, CrNiMo steel (1.4571)
- Gap between ATR probe and transducer approx. 1.4 mm (3 half-wavelengths)

Conclusion

With the ultrasound enhanced fibre optic probe it is possible to acquire spectra of yeast cells as well as the medium inline during fermentation. Changes in cell composition can thus be monitored quasi in real time; this could help to investigate cellular metabolism and detect possible limitations quickly. Successful acquisition of cell spectra was confirmed by comparison to spectra of dried cells.

Literature


The authors want to thank Pietro Palmesi for producing the technical drawings. Financial support was provided by the Austrian Science Fund (FWF) under the stand-alone-project theme (No. P24154). Cosima Koch acknowledges the financial support of Vienna University of Technology through the dedicated graduate school programme “Applied Bioscience Technology (ABTech)".