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Process Raman Spectroscopy for In-line Monitoring of Aerobic Fermentation of *Saccharomyces cerevisiae*

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Introduction

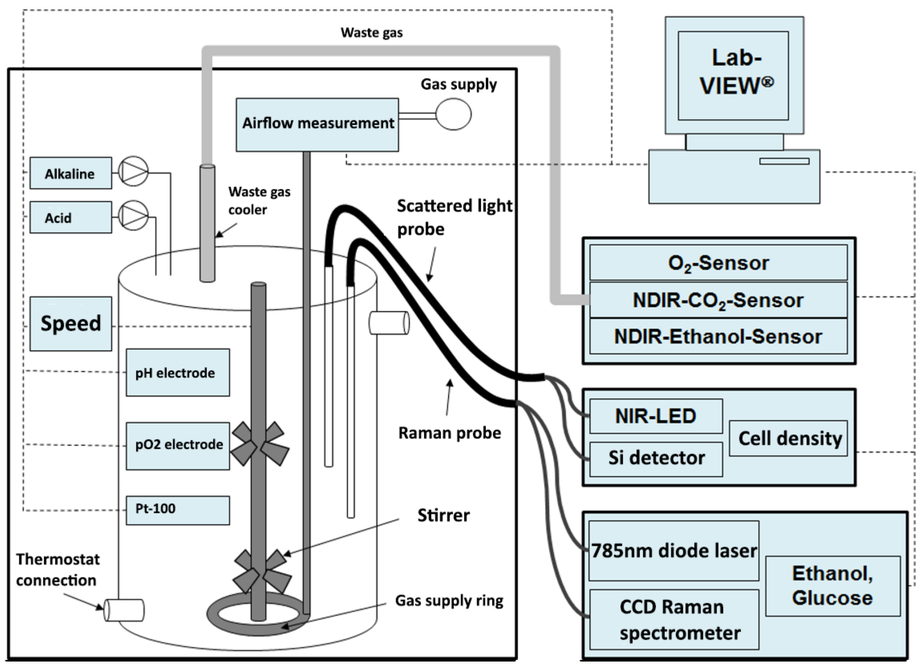
For automated process regulation in the chemical and biotechnology industries, measurement technologies are necessary which measure educt and produce concentrations in real time (in-line or on-line) and which also can be seamlessly integrated with the process-engineering environment. In recent years optical analytic methods have become increasingly dominant in this capacity. Optical spectroscopy allows rapid, non-invasive, and simultaneous determination of a number of analytes. Of the different optical spectroscopy technologies, MIR and Raman spectroscopy possess the greatest degree of selectivity, since the fundamental oscillations of molecules are stimulated.

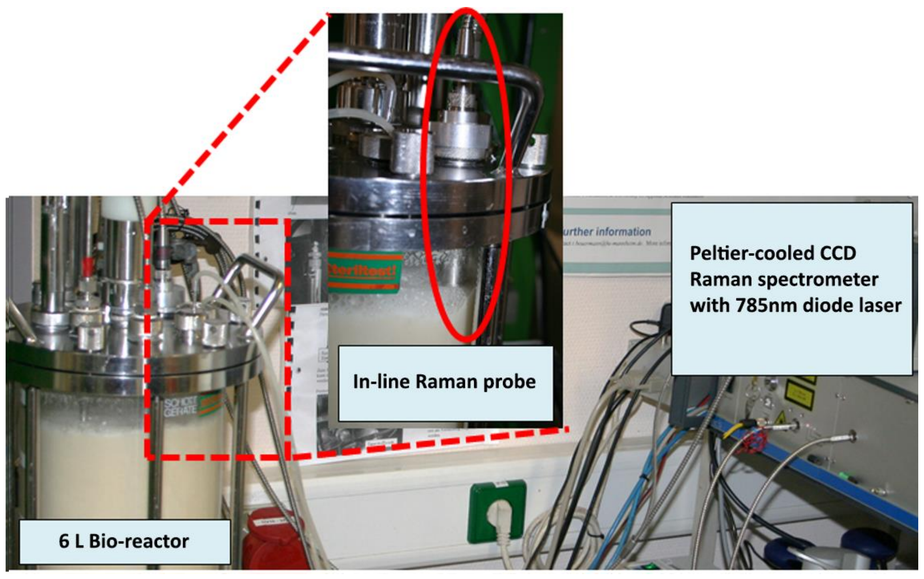
Raman spectroscopy

- Incident laser wavelength (inormally VIS or NIR light) is shifted to longer wavelengths (Raman shift)
- Combination of higher chemical specificity of MIR region using quartz VIS/NIR light guides
- Enables spatial separation of the measurement apparatus from the process
- More selective compared to NIR and UV/VIS spectroscopy
- Predestined for bio-process monitoring due to minimal disturbance from water

Measurement periphery and testing conditions

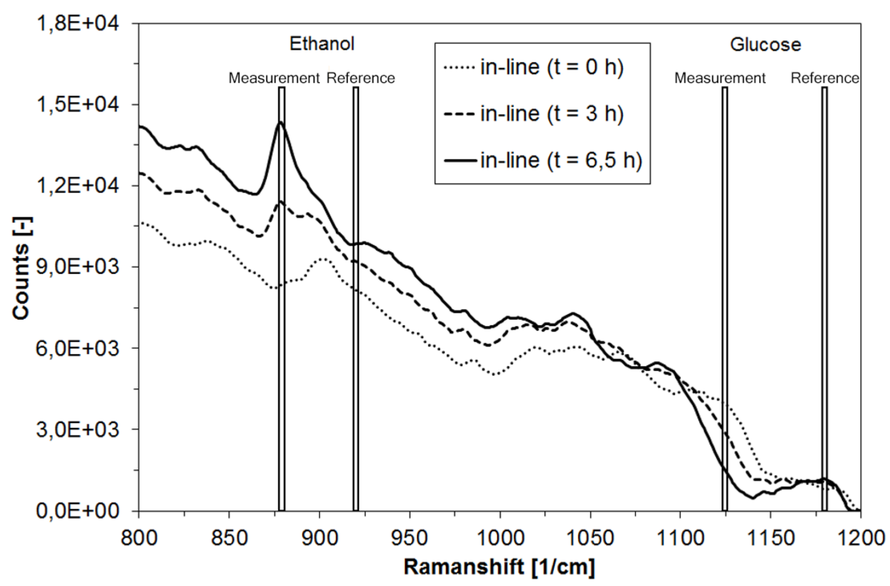
- 6 L bio-reactor (Biostat E, Fa. Sartorius); 5 L fermentation volume
- 100 g/L glucose as C source
- Inoculum: 33 g/L baker's yeast ≙ ca. 8.3 x 10^8 cells/mL (Fa. Wieninger)
- Gas application rate 1 vvm; pH 5.2; 32 °C; stirrer speed 700 rpm





Results

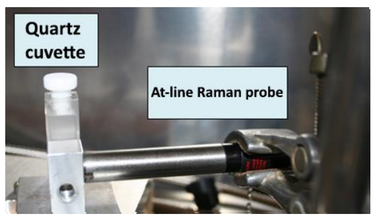
- Monitoring of substrate breakdown (glucose) and product formation (ethanol)
- Evaluation via the difference in middle Raman intensities from the measurement and reference range:

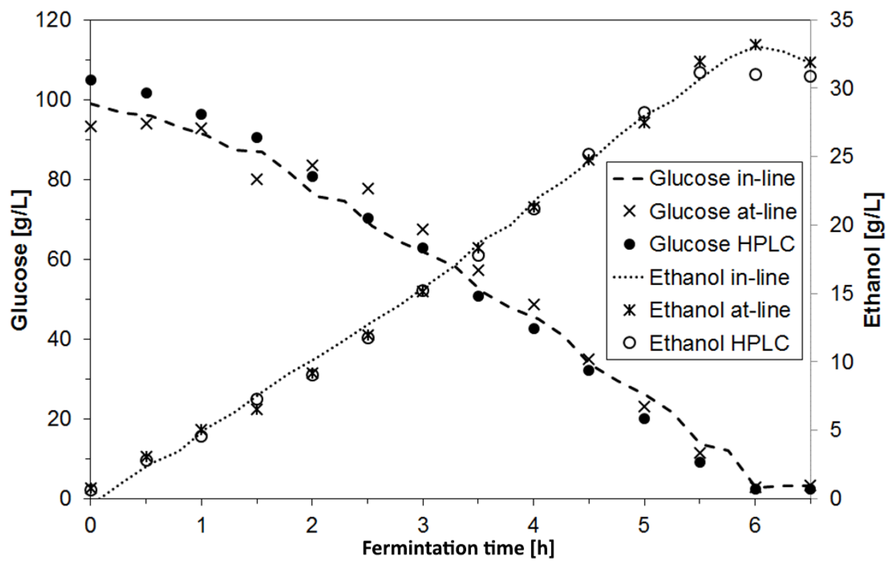


Analyte	Measurement range	Reference range
Ethanol	878 – 880 cm^{-1}	919 – 921 cm^{-1}
Glucose	1124 – 1126 cm^{-1}	1179 – 1181 cm^{-1}

Raman spectra of an aerobic batch fermentation of Saccharomyces cerevisiae incl. evaluation ranges of glucose and ethanol. Measurement with an in-line Raman probe in combination with a 785-nm diode laser and a CCD spectrometer (baseline adjusted to 1200 cm^{-1}).

- Additional quantification by at-line measurement of weighed material concentrations and fermentation tests





Monitoring of glucose and ethanol concentration in an aerobic batch fermentation of Saccharomyces cerevisiae. Comparison of in-line and at-line Raman measurements as well as external HPLC reference analytics.

Summary

- In-line monitoring of glucose and ethanol during yeast fermentation
- Simple evaluation by univariate calibration models
- Independent external validation by means of HPLC analysis

Contact

- For additional information, please write to **r.schalk@hs-mannheim.de**