Monitoring Yeast Fermentation Real-time using Raman Spectroscopy with Sapphire Ball Probe

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1. Background

Spectroscopy used in combination with multivariate data analysis methods enables concentrations to be monitored real-time during fermentation processes. Unlike MIR, Raman spectroscopy is less interfered by absorption from water and peaks are also more distinct than seen in NIR spectra. Recent developments of better detectors and probes designed for slurry liquids have further added to the benefits of Raman monitoring. However attenuation of Raman signal due to light scattering from yeast cells still complicates quantification. Effective scattering correction techniques can thus simplify and improve methods to determine concentrations using Raman.

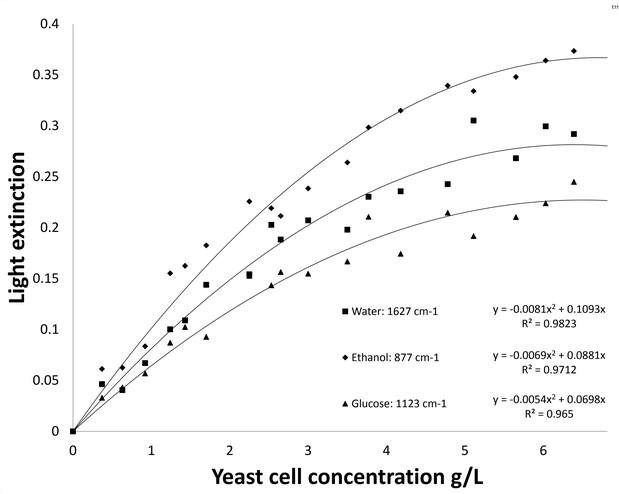
2. Objectives

- Study attenuation of Raman intensity due to scattering for use in correction method.
- Monitor concentrations of glucose, ethanol and yeast real-time using a simple quantification model not using chemometric methods.

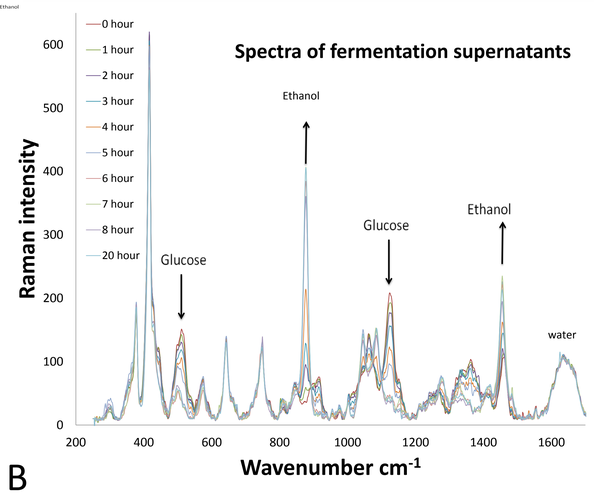
3. Methods

Correlation between glucose/ethanol concentrations and Raman intensity was found using synthetic media mixtures. An approximation method to correlate concentration of yeast cells and attenuation of Raman signal was made for initial scattering correction in quantification model. YPD medium with 40 g/L glucose and 3 g/L innoc. of *S. cerevisia* (Lallemand Ethanol Technology) was fermented at 35 C. ProRaman-I, 785nm excitation (Enwave Optronics Inc), equipped with immersion probe with ball sapphire lens was used for real-time in-situ measurements and compared with of-line OD and HPLC data.

4. Results



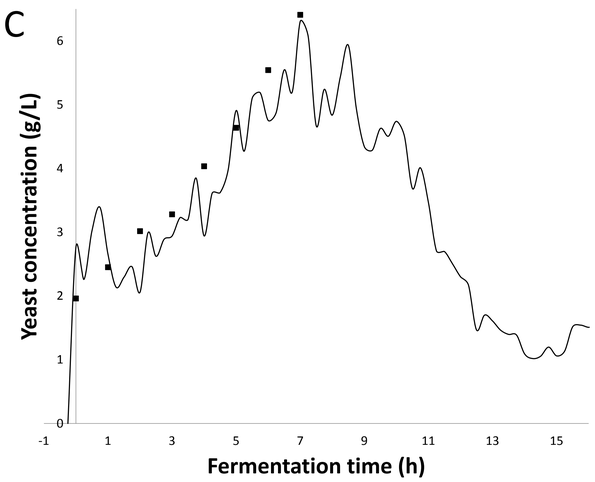
A



B

A. Non-linear extinction of Raman signals with increasing cells/scattering in glucose/ethanol mixture was approximated and used for scattering correction. B. Scattering correction was attempted by first determining cell concentration using water Raman signal at 1630 cm^{-1} as internal standard, which did not changed during fermentation. C. Predictions of yeast, D. ethanol and glucose concentrations was made upon scattering correction. Bioreactor stirring was stopped at 9h to see effect of scattering decrease due to cell precipitation.

C



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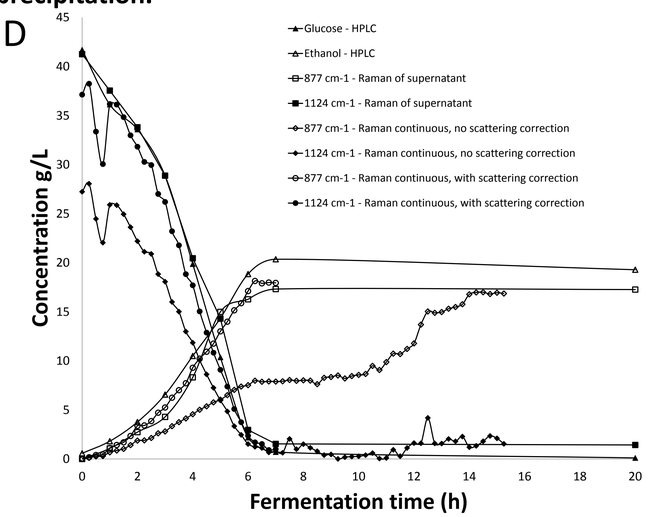

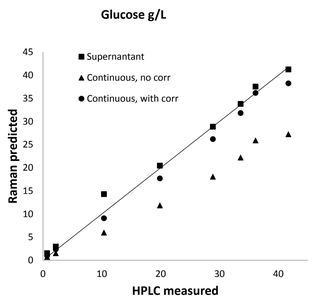

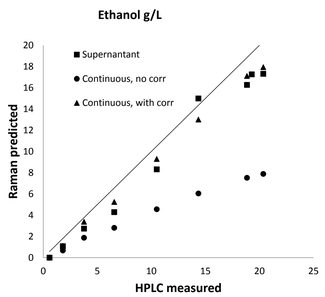

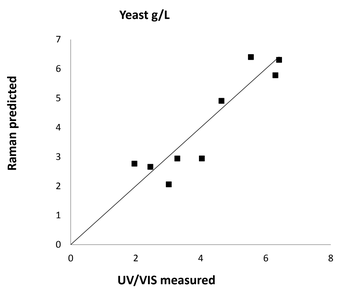

5. Conclusion

- Real-time measurement of fermentation process using Raman spectroscopy without complicated multivariate data analysis is possible using initial scattering correction.
- Better predictions have been achieved with commonly used chemometric methods such as PCA, but this simple quantification method eliminates the complicated "black box" where it is not needed.
- Using initial non-linear scatter correction in standard chemometric data analysis models could help to further improve accuracy of Raman measurements.

6. Future work

In extension to this work, Raman spectroscopy is currently being investigated as method to monitor:

- low temperature dilute acid pre-treatment of biomass
- hydrolysis of cellulosic material
- conversion during fermentation of pretreated and hydrolized lignocellulosic material.