Automated quantitative analysis of ethanol, glucose and NADH in the UV/VIS and short NIR

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Introduction

Process Analytical Technology currently becomes more and more important for food and pharmaceutical industry for example because of the increasing demand for more effective and efficient quality control. Therefore it is necessary to get timely and targeted responses of the different parameters in bioprocesses. To attain this aim, it is helpful to replace the conventional (manual) laboratory techniques with automated measuring processes via in-line probe.

There is a variety of probes and analytical techniques on the market for monitoring oxygen concentration, pH value, temperature or optical density. But similar automated analytical techniques are currently not available for a continuous measurement of other biotechnologically important substances like glucose, ethanol and NADH. In order to measure these substances directly and automated in the bioreactor, a suitable measurement system is required. For this purpose, in-line absorbance measurements have already been performed in the NIR [1] and MIR. Advantages of these wavelength ranges over the UV/VIS are the stronger absorbance (**figure 1**). However, the wavelength range from 400nm to 1000nm is also exciting due to the lower interfering water absorption and the low cost measurement technology based on silicon. Besides it is interesting due to the possible fluorescence of NADH.

The aim of this work will be to clarify whether it is possible to build up a system for automated in-line measurement of bioprocess parameters and to develop a calibration model for a prediction of concentrations via multivariate chemometric algorithms for UV/VIS and short NIR.

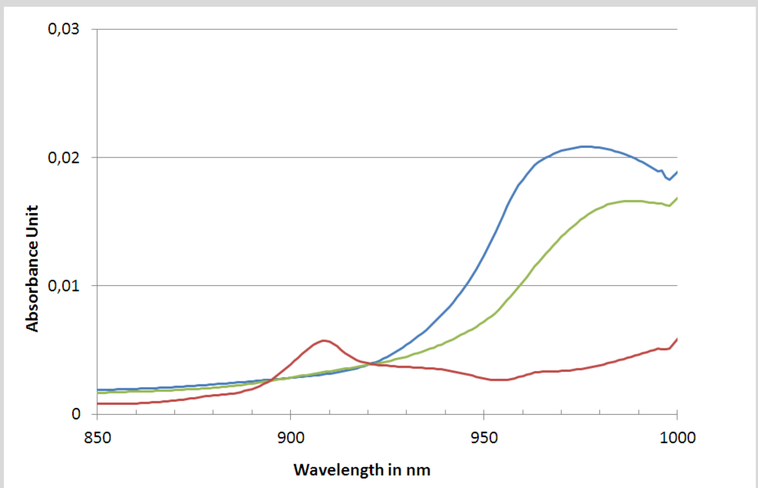

Figure 1: Absorption spectra of water (blue, measured), 100% glucose (green, calculated) and 100% ethanol (red, measured) in the short NIR.

UV/VIS and short NIR absorption spectroscopy and fluorescence spectroscopy

The UV/VIS system was built up with a CCD spectrometer with a wavelength range from 200nm to 980nm. The used transflectance dip probe (absorbance) or reflection probe (fluorescence) act as a direct interface between the UV/VIS system and the bioprocessing system. A second interface for a real-time process control could be realized via connection between the computer for chemometrics and the control unit of the bioprocessing system (**figure 2**).

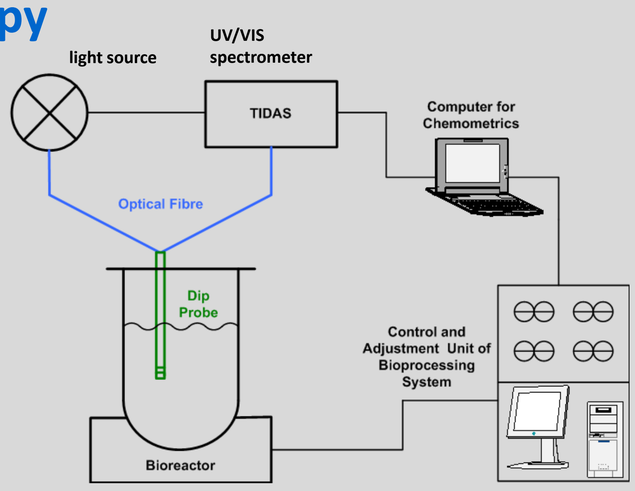

Figure 2: Integration of the UV/VIS system in the bioprocessing system via two interfaces.

Chemometrics

The application of statistical mathematical methods for the analysis of chemical data is called chemometrics. The complete chemometrics in this work was done via the software “The Unscrambler”. In combination with a PLSR algorithm (PLSR: Partial Least Squares Regression) different calibration models were generated (**figure 3**). Via the PLSR calibration models the concentrations of glucose, ethanol and NADH from the data of different UV/VIS spectra were predicted (**figure 4**).

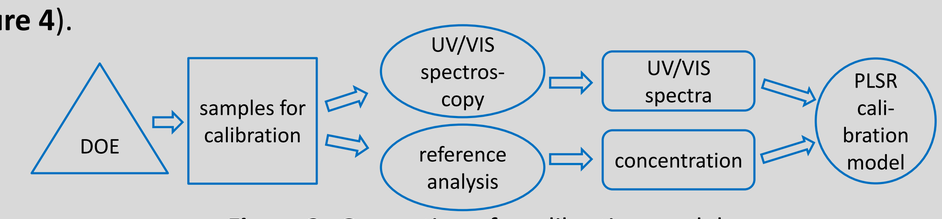

Figure 3: Generation of a calibration model

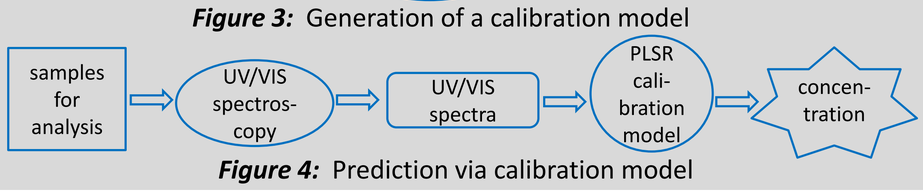

Figure 4: Prediction via calibration model

Preliminary tests

The preliminary tests were carried out with clear solutions of the analytes in water to determine whether ethanol and/or glucose show absorbance in the used wavelength range between 200nm and 980nm. Deionized water is used as reference. Due to the higher absorbance of water, the negative absorption in the wavelength range at about 970nm can be explained. Literature provides very contradictory information about these absorption peaks [2,3]. With the results in **figure 5** and **figure 6** we can confirm absorption peaks at about 910nm for ethanol and glucose.

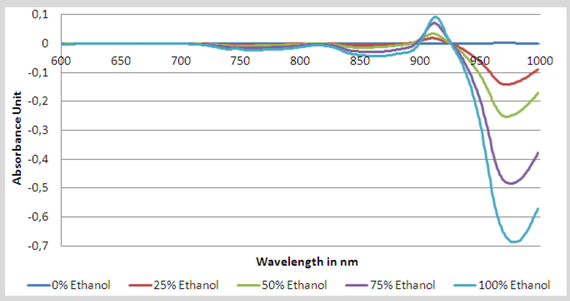

Figure 5: Ethanol absorption peak

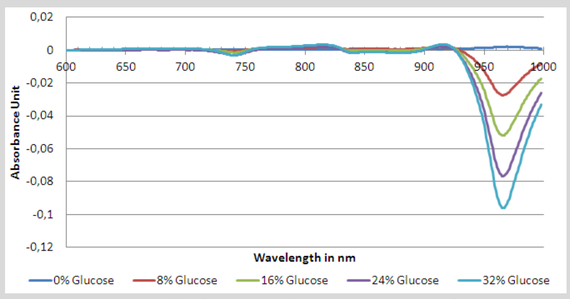

Figure 6: Small glucose absorption peak

Detection limit of ethanol in clear and turbid solutions and prediction

The lower detection limit is a value down to which it is possible to determine that indicators are reliable.

The calculation of the detection limit was carried out with the aid of DIN 32645.

The lower detection limit in clear solutions:

- clear solutions→ 3,8Vol.-% ethanol

The lower detection limit in turbid solutions:

- yeast concentration of 0,5g/L → 13,2Vol.-% ethanol
- yeast concentration of 1,0g/L → 15,1Vol.-% ethanol

It is not possible to determine ethanol concentrations in turbid solutions. In clear solutions it is possible but not very precise.

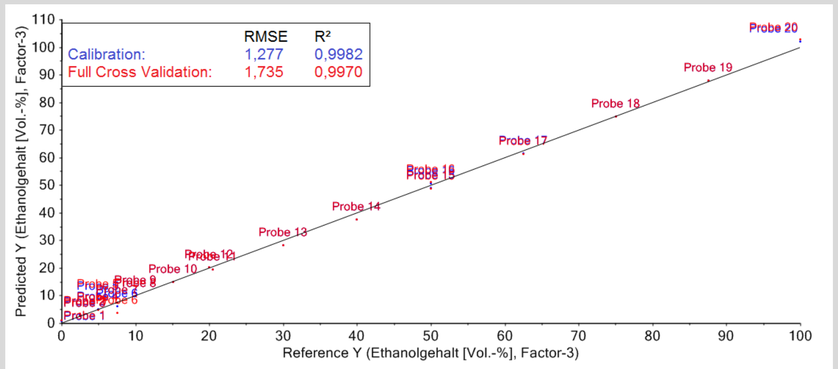

Figure 7: Comparison of predicted vs. reference value of ethanol in clear solutions

sample	ethanol concentration	
	predicted	weighed in
a	25,7%	26,6%
b	10,3%	10,0%
c	45,8%	46,6%
d	26,8%	28,4%
e	74,2%	62,3%

Quantitative determination of NADH via fluorescence

The NADH fluorescence intensity is an interesting parameter in bioprocesses. NADH is built during energy metabolism in a (yeast) cell by degradation of glucose.

So the NADH fluorescence is dependent on the yeast cell mass and the glucose supply.

But currently there is no measurement technology available on the market to use fluorescence for quantitative determination of NADH.

Here is an approach which shows the production of NADH-Na_2 solutions in water as a reproducible method for generating desired NADH concentrations and automated quantitative determination of NADH concentration via fluorescence spectroscopy. The lower detection limit is about 0,289mg/L in clear solutions. In **figure 8** the different fluorescence intensities and NADH concentrations are shown.

It is also probably possible to determine NADH in turbid solutions in the bioreactor, but no predictions were made yet. With increasing yeast cell mass, the fluorescence of NADH gains as long as there is glucose available. This correlation is shown in **figure 9**.

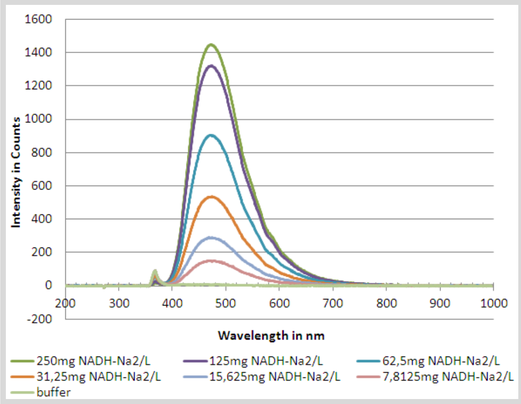

Figure 8: NADH fluorescence peak at 470nm, determined by NADH-Na2 solutions

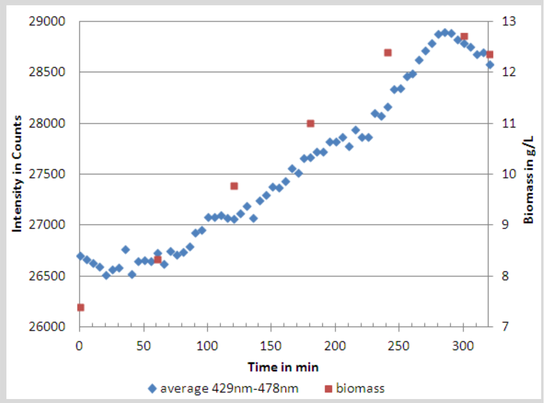

Figure 9: Correlation between NADH fluorescence and yeast cell mass during an anaerobic yeast fermentation

Conclusion

The automation of the measuring process in the UV/VIS and short NIR is possible only to a limited degree. For glucose it wasn t successful. Ethanol concentrations can only be predicted in clear but not in turbid solutions. But as shown in the thesis of Princz (University of Applied Sciences, Ulm), we have the possibility to predict both in the NIR [1].

With rising NADH concentration also the fluorescence intensity increases until a concentration of about 250mg NADH-Na_2/L is exceeded. In the upper concentration ranges the fluorescence intensity decreases probably due to self-absorption. For NADH solutions with low NADH concentrations it is possible to determine a correlation between fluorescence intensity and yeast cell mass during an anaerobic yeast fermentation.

References

[1] S. Princz, Inline-Bestimmung des Ethanol- und Glucosegehalts in einem Laborfermenter mit Hilfe der NIR-Spektroskopie und multivariater Datenanalyse, Ulm, Master-Thesis, 2012

[2] F. D. Barboza und R. J. Poppi, „Determination of alcohol content in beverages using short-wave near-infrared spectroscopy and temperature correction by transfer calibration procedures,“ Anal Bioanal Chem 377:, pp. 695-701, 2003

[3] J. D. Manuell, A Simulation-Based Study on the Application of Artificial Neural Networks to the NIR Spectroscopic Measurement of Blood Glucose, Johannesburg, Master-Thesis 2008

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