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Mid Infrared (MIR) Transmission Spectroscopic Methods for Aqueous Samples

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Mid Infrared (MIR) Transmission Spectroscopic Methods for Aqueous Samples - Monitoring and characterization of bioprocesses

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The ability to rapidly and precisely determine multiple process parameters with simple and reliable methods is highly important in biopharmaceutical research and development.
An online technology is thus required that can deliver dependable and device independent data. This article presents a new approach to the use of mid infrared range (MIR) transmission spectroscopy in the context of bioprocess engineering.

Although advances in biopharmaceuticals have gained steadily in importance in recent years, real-time measurement methods are still largely lacking in the area of bioprocess engineering. Spectroscopic methods tend to have the greatest potential in terms of filling these gaps, but no such methods established themselves to date.

Methods employed hitherto such as near infrared range (NIR), Raman or attenuated total reflection (ATR) do not satisfy the pertinent requirements. NIR and Raman do not cover the required analytical spectrum or show weak interaction in the corresponding spectral range [1], [2]. Disadvantages associated with ATR include: the absorptions are wavelength-dependent and interface effects can occur between optical material and liquid sample, for instance in the form of adsorption or denaturation of the sample [1], [3], [4]. There is also a problem concerning the transferability of the spectral databases and/or the chemometric models from device to device [5].

Fig. 1: Automated MIRA Analyzer

The use of MIR spectroscopy (spectral range from 4000 cm^{-1} to 400 cm^{-1}) to monitor the manufacturing processes and to screen active substances is now a standard method used in the chemical and pharmaceutical industry because of the high informational content of the spectra. Until now, however, this method could not be used for aqueous samples. This is due to the strong absorption of water in the mid infrared range and related total absorption. The technological requirements for MIR systems for use with aqueous samples are very high, i.e. light paths in the micrometer range and light path accuracy in the nanometer range.

New interface technology MIR measuring techniques

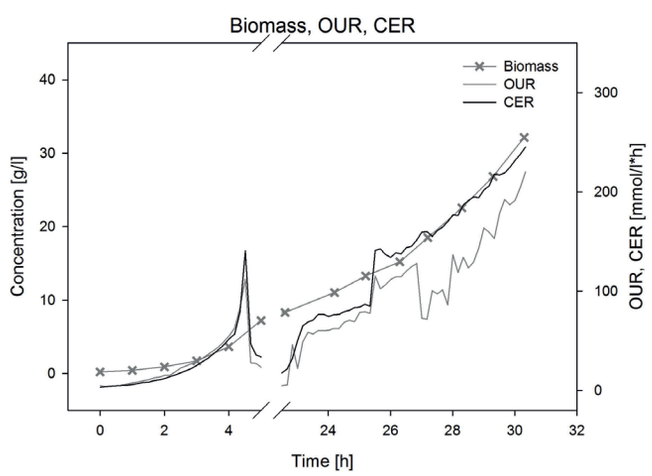
Flow-through cells to measure transmission are common in infrared spectroscopy. Liquid test samples are directed through such cells using inlet and outlet channels and passed through an optical measuring chamber, which is separated by infrared-transparent windows. The MIR transmission spectroscopy methods presented here are performed with a precision flow cell manufactured using microsystems technology, which use defined light paths of under 10 µm and are pressure resistant. The measuring cell ensures light path accuracy better than 1 nm. **This special flow cell design allows for the reliable analysis of aqueous samples** (http://micro-biolytics.com/en/technologie/). The full potential offered by mid infrared spectroscopy can now be realised in combina-

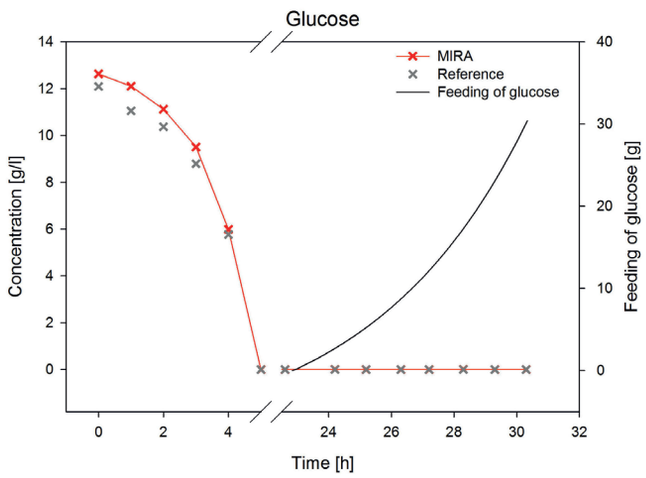
tion with multivariate methods of analysis for aqueous samples. Analyses are possible in the lower ppm range (detection limit: 5 – 10 mg/l), what is required in bioprocess-related analytics in particular. The short analysis times of only a few minutes and low sample volumes (less than 100 µL) allow simultaneous and continuous quantification of many process-relevant analytes in form of at line & online integration. In addition to the quantification of substances, MIR technology also facilitates the determination of protein structures and conformational states.

The analysis of chemometric data serves to detect unknown effects, indirect parameters and quality-relevant process factors. **With database-supported algorithms calibration is no longer required.** This plays a particularly important role in the development of bioprocesses, because there are many variations in terms of media composition and process control to be monitored. Classically calibrated chemometric models (e.g. PLS-R Partial Least Squares Regression) necessitate recalibration for each change in media composition. Due to the considerable effort required for each calibration, these methods are not employed in optimising and developing bioprocesses.

Using MIR transmission spectroscopy for process analytics during cultivation of *E. coli*.

MIR transmission spectroscopy shows great potential for the cultivation of *E. coli* in the multi-bioreactor system. In such cultivation processes, the accompanying analytics were conducted via MIR technology, with the exception of biomass concentration. The fully automated MIRA Analyzer (Fig. 1, http://micro-biolytics.com/en/technologie/produkte/analyzer/) is based on a PAL System (www.palsystem.com). For the quantitative analysis of culture media components such as glucose, ammonia, phosphate, sulphate, citrate and proline, a calibration-free predictive algorithm has been developed. In addition to the most important culture media components, the byproducts acetate, lactate, succinate and pyruvate were also determined on the basis of this predictive model. The most significant byproduct, acetate, is formed by *E. coli* under aerobic conditions in the presence of excess glucose and impairs cell growth and product formation. Analysis of the acetate concentration over time therefore contributes significantly to efficient process control and has so far not been available as a parameter measured online. To check the predictive algorithm, the results of analysis are compared to classical reference analytics obtained from MIR transmission spectroscopy (Fig. 2).





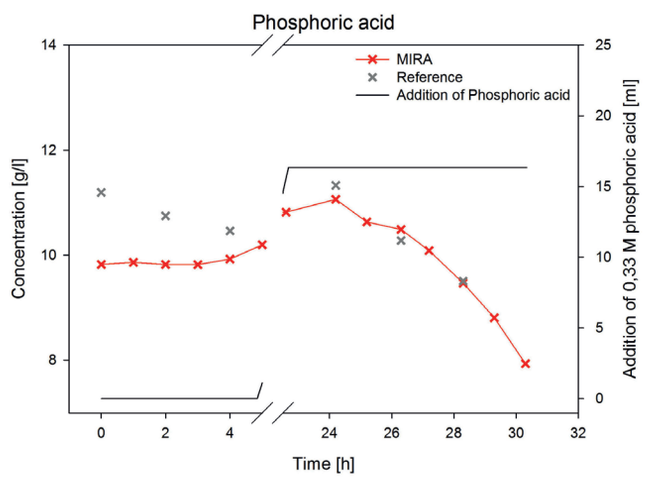


Fig. 2:
Process analytics of glucose, acetate and phosphate with MIR transmission spectroscopy during cultivation of E. coli TB1 pGLO. The cultivation was performed with an exponential fed-batch profile in a multiple bioreactor system. 0.33 M phosphoric acid and 25% ammonia were used to regulate pH. The reference analyses of glucose, acetate and phosphoric acid were carried out using an enzymatic test kit. OUR and CER measurements were based on an exhaust gas analysis. For operational reasons the cultivation process had to be interrupted for approximately 20 hours, the fed-batch phase was begun approx. 22 hours later.

Outlook

MIR technology offers shorter measuring times, less time-consuming sample preparation, complete digitisation of results and high information content. MIR technology opens the door to a range of innovative applications, while also offering significant benefits in terms of saved time and costs. As the use of MIR transmission spectroscopy in the example above has shown, the method can realise its full potential in the area of bioprocess development. Rapid and comprehensive analysis and the possibility of linking multiple bioreactors to an automated measuring system enable efficient screening procedures. The holistic determination of process parameters is a prerequisite for modern quality-by-design (QbD) approaches that require a deeper understanding of the entire process. As opposed to currently available methods such as HPLC or MS, MIR transmission spectroscopy does not require complex assessment techniques from the end users. The continuous expansion of the substance databases for the predictive algorithm will make it possible to determine many more substances in the future, without having to establish further analytical methods.
The complete automation of the analytical process with the MIRA Analyzer greatly increases process safety and productivity.

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Project funded by Bundesministerium für Wirtschaft und Energie by a joint resolution of the German Bundestag.

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Imprint

Date of print: 04.2016

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