**Photon Density Wave spectroscopy for in-line monitoring of biomass in high-density fermentation processes**

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**Highlights**

* In-line Biomass measurements via Photon Density Wave spectroscopy
* Yeast and bacterial cultivations up to 67 g L-1
* Separate quantification of scattering and absorption properties
* Detection of process deviations

**1. Introduction**

The ability to monitor and control the formation of biomass during industrial bioprocesses is of high interest as critical factors like maximal biomass production, the physiological state of cells, product formation rate, and product quality are dependent on the specific growth rate [1]. Especially at high cell densities or in very turbid and disperse fermentation media established optical process analytical technologies face signal saturation effects or are prone to probe fouling. In this contribution Photon Density Wave (PDW) spectroscopy will be introduced as an in-line analytical tool for monitoring and control of bacterial and yeast cultivations.

A fully autoclavable and chemically sterilizable, calibration-free process analytical technology suitable to highest concentrations (i.e. > 40 vol%) in stirred or flowing systems is found in Photon Density Wave spectroscopy [2][3]. It allows for the independent quantification of the absorption and scattering properties of disperse materials, i.e. the absorption and the reduced scattering coefficients, respectively. The absorption coefficient exhibits information about concentration changes of light absorbing media components during the investigated process. The reduced scattering coefficient, however is related to the size and concentration of the dispersed particles such as cells.

**2. Methods**

Fed-batch cultivations of *Escherichia coli* and *Kluyveromyces marxianus* were performed in 3.7 L and 10 L bioreactors respectively using both defined and complex media while focusing on high biomass concentrations. Optical density and cell dry weight (CDW) were obtained by offline sampling. As reference PDW spectroscopy was implemented to track the biomass formation in-line and its performance was compared to other in-line methods, such as turbidity and backscatter measurements.

**3. Results and discussion**

The reduced scattering coefficient *µs’* obtained by PDW spectroscopy proved to be a reliable indicator for the respective biomass concentration during reactor cultivations. Especially at high turbidities correlation coefficients of R2 = 0.99 between *µs’* and CDW were obtained, while the reference methods showed non-linear responses driven by saturation effects.

More comprehensive understanding of the process could be generated by investigating the simultaneously measured absorption coefficient *µa* in relation to *µs’*. This way process disturbances induced by e.g. inadequate control strategies were detected in-line and early into the cultivations.

**4. Conclusions**

First investigations show Photon Density Wave spectroscopy to be a promising technique for the monitoring of stirred tank reactor cultivations. It enables the real-time measurement of biomass formation under process conditions without the indication of concentration-induced saturation effects.

**References**

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