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Investigation of different methods for quantitative microbial biomass monitoring in microbial fermentations using Raman spectroscopy

In-line Raman spectroscopy combined with chemometric modelling represents a powerful process analytical technology (PAT) for real-time, quantitative monitoring of microbial fermentations. While Raman-based models have been successfully applied to quantify key chemical components [1], the development of robust models for biomass monitoring remains relatively unexplored. Furthermore, the transferability of Raman-based biomass quantification across different fermentation conditions and microbial species has not been systematically investigated.

In this study, biomass quantification models were developed using two distinct Raman-based approaches: (i) spectral features associated with biomass chemical composition (feature-based approach) [1] and (ii) particle-induced signal attenuation (attenuation-based approach) [2]. For each approach, the effects of growth phase, sample temperature, suspension medium and microbial species (*Escherichia coli* and *Saccharomyces cerevisiae*) on predictive performance were evaluated. Models were constructed to predict optical density (OD) and cell dry weight (CDW).

Preliminary results demonstrate differences in model transferability between attenuation-based and feature-based approaches. The observed variability is likely linked to differences in biomass composition, particularly protein content, between species and physiological states. In addition, both growth phase and extracellular matrix composition influenced Raman-based biomass predictions. Because these characteristics change dynamically throughout fermentation, they can adversely affect model robustness and predictive accuracy.

These findings highlight the importance of understanding the spectral changes induced by biomass and its physiological state when developing transferable Raman calibration models. Accounting for biological and process-related variability may improve model robustness, enhance transferability across fermentation conditions and species, and reduce calibration effort. Overall, this work contributes to the development of a broadly applicable PAT platform for real-time biomass quantification in microbial fermentations using Raman spectroscopy.

References

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- [2] Yang, N., Guerin, C., Kokanyan, N., & Perré, P. (2024). In-line monitoring of bioreactor by Raman spectroscopy: Direct use of a standard-based model through cell-scattering correction. *Journal of Biotechnology*, 396, 41-52.