In-line Dielectric and Raman Monitoring of Intracellular Lycopene Production in *E. coli* Fermentation Scale-Up

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Abstract

The use of in-line analytics for monitoring and understanding biological processes has been promoted since 2004 through the Process Analytical Technology (PAT) initiative. Two such technologies are Raman and dielectric spectroscopies that are well established but deployed independently. For the very first time, simultaneous usage of in-line Raman and dielectric spectroscopic analytics for tracking progress of a lycopene overproducer *E. coli* 322 engineered strain was studied herein, alongside scale-up experimentation from shake flask to 0.5L and 10L bioreactor cultures. An intracellular lycopene extraction protocol was optimised for off-line quantification by Raman or UV-Vis spectroscopies, which aids the estimation of lycopene over-production in a high cell density *E. coli* fed-batch culture via in-line Raman. Correlating dielectric capacitance recordings and flow cytometry viable cell count measurements allows for real-time cell culture viability monitoring, which is more informative for bioprocess dynamic control in contrast to conventional in-/off-line biomass optical density (OD_{600}) or dry cell weight (g_{DCW}). This enabled fed-batch culture revitalisation prior to inducing intracellular lycopene production. The final biomass OD_{600} was ca. 81.8 corresponding to in-line dielectric capacitance estimated culture viability 43.38 g_{VCC}/L. Intracellular lycopene yield was estimated at 495.1 mg/g_{VCC} via in-line Raman, which was 12.3 times increase compared to initial trial reported for *E. coli* 322. The successful implementation of dual in-line dielectric capacitance and Raman monitoring exemplifies the quality by design (QbD) approach espoused by PAT framework for expediting bioprocess understanding, development and scale-up of engineered microbial strains.

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