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Single cell analysis and its application to bioprocess characterization, monitoring and design

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Microorganisms in industrial processes are considered conventionally as uniform populations and therefore are thought to be sufficiently described by integral values of process variables. Particularly for fed batch processes where high cell densities can be reached, it was shown in the past that this is not true[1,2,3,4]. For example, reduction of up to 20% of cell viability within the population was observed during fed-batch processes with *E. coli*[5]. This situation highlights the need of reliable methods to adequately characterize and monitor bioprocesses for production intensities at single cell level occurring, e.g. under substrate-limited or high cell densities cultures.

Illustrated at the example of the antibody fragment D1.3 scFV production, several batch and fed-batch cultivations of a recombinant *Bacillus megaterium* strain were performed. Thereby, different dyes for viability estimation considering membrane potential and cell integrity were successfully evaluated for *B. megaterium* single cell characterization. Moreover, it was possible to establish an appropriate assay to measure the production intensities of single cells revealing not only the location of product secretion, but also its dynamics. By these means it was possible to distinguish between live, metabolic active, depolarized, dormant, and dead cells and to discriminate between high and low productive cells. Cell integrity, membrane potential and production intensity measurements revealed clear changes in the cell status at the transition from exponential to stationary phase in batch cultivations. Depolarization of cells in the stationary phase was accompanied with decreased membrane integrity, a phenomenon that may be directly related to the increase in production and a delayed secretion of antibody fragment D1.3 scFv due to changes in the cell structure based on higher autolysin activity, which depends on the cell wall charge distribution[1]. Therefore, the easy principle of late induction for improved production was not directly applicable in the process, making compulsory the implementation of a µ-oscillating adapted fed batch strategy, where sequential growth/production-secretion phases were alternated. The approach could be established at the lab scale and was successfully scaled-up to pilot scale using a 100L-bioreactor. First hints on the reason why lower overall productivities were reached at 100L-scale compared to lab scale were found using the so-called scale-down experiments with GFP as recombinant model protein. The studies, used to mimic large scale behavior, reveled that certain intracellular amino acids oscillated together with the main substrates, but were never limiting to recombinant protein production. Contrastingly, others amino acids were completely consumed after initial oscillation and formed a metabolic bottleneck until the end of the cultivations. The simply supplementation of the limiting amino acids in the fresh feed medium eliminated the metabolic bottleneck effectively.

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