

## Functionality of microbial phenotypic heterogeneity in bioprocessing conditions: an analysis based on the use of on-line flow cytometry

Microbial phenotypic heterogeneity is known to be naturally present in isogenic population and can be attributed to the stochastic nature of the biochemical reactions. An important question at this level was to determine whether such stochastic behavior exhibits some functionality, i.e. how single cell heterogeneity leads to population level strategies. One of this strategies, called bet-hedging, is known to give a competitive advantage to the population, by leading for example to a persistent phenotype able to survive to antibiotics exposure. Among the single cell toolbox available for the analysis of phenotypic heterogeneity, flow cytometry present the advantage of being compatible with bioprocess cultivation tools. In this work, we present an on-line analytical workflow based on automated flow cytometry that can be used to monitor simultaneously several bioreactors, testing different bioreactor operating conditions, in combination with fluorescent transcriptional reporter strategies: promoter involved in metabolism fused to a gene expressing an unstable variant of GFP , and viability fluorescent tagging: propidium iodide uptake, correlated with membrane permeability.