Scaling-up With the Eyes of the Microbe: Lessons to be Considered for Implementing Robust Strains and Processes

Löffler M., Simen J., Michalowski A., Niess A., Ziegler M, Zieringer J., Ankenbauer A., **Takors R.**
Institute of Biochemical Engineering, University of Stuttgart, Allmandring 31, 70569 Stuttgart, Germany
takors@ibvt.uni-stuttgart.de

For commercialization, strain and bioprocess developments need to be successfully transferred from the lab to industrial scale. Often, this step crucially decides about economic feasibility and survival of the approach. Accordingly, profound understanding of impact factors that hamper the successful scale-up is key, either to create novel microbial production platforms with enhanced robustness or to improve bioreactor design targeting minimized impact on cellular performance.
Using an experimental scale-up *Escherichia coli* was exposed to typical large-scale mixing conditions in continuous experiments. Installing mixing times of about 110 seconds and simulating fluctuating availability of carbon and nitrogen sources, short-term responses revealed the repeated on/off switching of about 600 genes. Dynamics of gene expression and protein formation were modelled using an agent-based approach for simulating large-scale conditions. ATP balancing of gene expression and protein formation showed that maintenance demands increased by ~50%. Thereof, strategies for genome reduction were deduced.

Because transcriptional regulation was dominated by the on/off switching of stringent response, metabolic engineering was performed to keep intracellular *ppGpp* levels, i.e. the pool sizes of the alarmone, on non-perturbed levels. It will be shown that the said strain *E. coli SR* minimizes the transcriptional stimuli in large-scale simulators down to a 1/7 of wild-type responses. Thereof, *E. coli* HGT (high glucose throughput) was derived enabling maximized glucose input even under resting and slow-growth conditions. Large-scale modelling with Euler-Lagrangian approaches links the experimental observations with the prediction of cellular performance in industrial stirred tank reactors.

The approach has been applied further using *Pseudomonas putida* KT2440, a promising candidate for industrial applications. Whereas basic transcriptional dynamics were similar to *E. coli*, fundamental differences became obvious thanks to unique counter measures taken by *P. putida* in large scale bioreactors.