**Designing scale down fermentations: strategies and challenges**

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**Highlights**

* Scale down simulator design based on CFD-CRD model
* Following life lines of organism, seeing through microorganism view
* Microfluidics techniques studying single cell and population heterogeneity

**1. Introduction**

In microbial fermentations, it is frequently observed that upon scaling up of processes there is a loss in performance (yields/productivity/titers) due to lack of homogeneity in industrial fermentors. It is now generally recognized that a good scale down (SD) protocol is necessary to capture the inhomogeneities (for C-source, oxygen, precursors, pH, temperature, or shear stress) due to realistic mixing times at production scale. Depending on the characteristic times, one or more of these gradients may have impact on the performance of strain/process in fermentation. Developing a scale down protocol for lab-scale environment that reflects industrial heterogeneities is challenging due limited knowledge of said heterogeneity. Traditionally, a scale down protocol mimicking the heterogeneity based on characteristic times alone is insufficient as this method lacks spatial resolution.

**2. SD simulator design approach**

An alternative solution that was proposed was a scale down approach using a novel methodology integrating computational fluid (CFD) and reaction dynamics (CRD) modelling of industrial fermentors [1]. In these simulations, the multiphase flow and transport phenomena taking place inside the bioreactor are simulated and integrated with the microbial reaction kinetics (e.g. substrate uptake) [2]. The microorganisms inside the reactor are followed along their trajectories, where they experience a changing environment. This approach resulted in metabolic response from the microorganism point of view and provided input for experimental design of scale down simulators.

**3. Strategies and Challenges**

This CFD-CRD coupling is an improvement over the traditional approach for designing scale down experiments, though it brings its own challenges that need to be addressed [2]. On the CFD side, the bioreactor hydrodynamics such as macro-mixing, gas–liquid interaction, and microbial reactions are often considered dominant. However, for specific cases it may be necessary to consider to look into meso-mixing, eddy micro mixing, film diffusion for viscous processes, including non-Newtonian rheology. On the CRD side, currently, the models coupled with CFD are limited to metabolic models but that can be extended to a broader spectrum of cellular responses (eg. transcription and translation dynamics, protein formation, cell cycles), provided the resolved timescales are sufficient to capture such dynamics. Furthermore, the CRD models should be designed by keeping its coupling with CFD in mind such as to avoid instabilities eg. noisy rates or non-zero rates at zero pool size. Computational advances are required to enable full fermentation duration, and real-time simulations.

Actual design of SD simulators is guided by the lifeline structure (smooth changes versus discrete jumps) and practical considerations. In a single vessel SD-simulator, which is straightforward to operate and analyse, all organisms see the same extracellular environment and the effect of extreme fluctuations cannot be captured. On the other hand, a multi-vessel simulator accounts for a distribution of the exposure time to the various conditions by design, but not for the distribution of the amplitude. Additionally, the operational challenges will remain such as pumping issues for shear-sensitive and complex rheology systems. While current SD simulators operating at industrial biomass concentration may capture dynamics at the average level, more rapid dynamics at the individual cell level are unattainable. Some of these challenges cannot be overcome in single system and the selection of the SD simulator will depend on the case study at hand. It was shown that single and multiple compartments do not show similar microbial response for identical extracellular disturbances [4].

The challenges still remain for the SD simulators such as to decouple rate-of-change from consumption, to impose the full range of amplitudes and (stochastic) durations, and that each cell undergoes a different experience similar to the industrial scenario. Some of these can further be tackled using advancements in microfluidic devices, using fluorescent techniques.

**4. Conclusions**

Integrating computational fluid (CFD) and reaction dynamics (CRD) modelling of industrial fermentors allows improved design of SD simulators. Some of the challenges remain such as improving the simulation of bioreactor hydrodynamics, coupling of CFD-CRD models, and lack of industrial scale data for model validation. Some of the issues can be solved by increasing computational power, efficient CFD-CRD models, and scaling down further to microfluidics level.

 **References**

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