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Editorial

Special Issue "Bioprocess Systems Engineering Applications in Pharmaceutical Manufacturing"

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Biopharmaceutical and pharmaceutical manufacturing are strongly influenced by the process analytical technology initiative (PAT) and quality by design (QbD) methodologies, which are designed to enhance the understanding of more integrated processes. The major aim of this effort can be summarized as developing a mechanistic understanding of a wide range of process steps, including the development of technologies to perform online measurements and real-time control and optimization. Furthermore, minimization of the number of empirical experiments and the model-assisted exploration of the process design space are targeted. Even if tremendous progress has been achieved so far, there is still work to be carried out in order to realize the full potential of the process systems engineering toolbox.

Within this Special Issue of *Processes*, an overview of cutting-edge developments of process systems engineering for biopharmaceutical and pharmaceutical manufacturing processes is given, including model-based process design, Digital Twins, computer-aided process understanding, process development and optimization, and monitoring and control of bioprocesses. The biopharmaceutical processes addressed focus on the manufacturing of biopharmaceuticals, mainly by Chinese hamster ovary (CHO) cells, as well as adeno-associated virus production and generation of cell spheroids for cell therapies.

Both model-based process designs and the Digital Twin concept have gained increasing interest for the development and optimization of biopharmaceutical production processes. Such methods are still not state-of-the-art for cell culture processes during development or manufacturing, although first approaches have been proposed. This highlights a need for improved methods and tools for optimal experimental design, optimal and robust process design, and process optimization for the purposes of monitoring and control during manufacturing. Three contributions within this Special Issue address this topic, which are highlighted as follows:

Bayer et al. [1] present a digital bioprocess twin used for a model-based design of experiment (DoE) to accelerate the design space exploration and thereby decrease the time needed to identify the optimum combination of critical process parameters (CPP) for the variables of interest. This Digital Twin simultaneously delivers additional process understanding while accelerating bioprocess development and optimization by applying in silico simulations and only perform the recommended experiments. A structured workflow is presented using different initial data sets to reduce experimental efforts, evaluate the results, and additionally to investigate the applicability of an intensified DoE (iDoE) for such a model-based DoE. By this, the best CPP combinations in a design space are identified with the highest space-time yield.

Strategies for multi-objective optimization of industrial biopharmaceutical processes are addressed by Hernández Rodríguez et al. [2]. In industrial applications, it is typically desirable to optimize several conflicting objectives at a time, leading to suitable trade-offs and compromises. However, multi-objective optimization is more complex and its application is still not state-of-the-art in the context of cell culture processes (probably

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due to a lack of related studies and instructions). The contribution presents a conceptual workflow which couples uncertainty-based upstream simulation and Bayes optimization using Gaussian processes. Its application is demonstrated in a simulation case for the design of a robust cell culture expansion process (seed train), meaning that despite uncertainties and variabilities concerning cell growth, low variations of viable cell density during the seed train are obtained. This approach provides the potential to be used in the form of a decision tool (e.g., for the choice of an optimal and robust seed train design or for further optimization tasks within process development).

The selection of appropriate clones of production cells is essential for the optimized manufacturing of complex biopharmaceuticals using bioreactors. A simulation study on model-assisted clone selection for CHO-cells is presented by Hernández Rodríguez et al. The authors of [3] address the question, if clonal cell populations showing high cell-specific growth rates are more favourable than cell lines with higher cell-specific productivities (or vice versa). A mechanistic cell culture model was adapted to the experimental data of such clonally-derived cell population. Uncertainties and prior knowledge concerning model parameters were considered using Bayesian parameter estimations. This model was then used to define an inoculum train protocol. It could be shown that growth rates have a higher impact on overall process productivity and for product output per year, whereas cells with higher productivity can potentially generate higher product concentrations in the production vessel.

Besides the use of mechanistic process models, computational fluid dynamic studies can support bioprocess design and optimization. One paper in particular focuses on *computer-aided process understanding*. Bioreactor design and scale-up in today's biopharma industry relies mostly on empirical correlations, experience, and engineering heuristics, which can hardly provide the link between hydrodynamics within the bioreactor and biological process behaviour. Freiberger et al. [4] investigated cellular effects and locally resolved hydrodynamics in stirred bioreactors for impellers with different spatial hydrodynamics. Therefore, the hydrodynamics, mainly flow velocity, shear rate, and power input in a single- and a three-impeller bioreactor setup were analyzed by means of CFD simulations, and cultivation experiments with antibody-producing CHO cells were performed at various agitation rates in both reactor setups. It could be shown that behaviour of the cells in the different reactor set-ups cannot be linked to parameters commonly used to describe shear effects on cells such as the mean energy dissipation rate or the Kolmogorov length scale, even if this concept is extended by locally resolved hydrodynamic parameters. Alternatively, the hydrodynamic heterogeneity was statistically quantified by means of variance coefficients of the hydrodynamic parameters fluid velocity, shear rate, and energy dissipation rate. The calculated variance coefficients of all hydrodynamic parameters were higher in the setup with three impellers than in the single impeller setup, which might explain the rather stable process behaviour in multiple impeller systems due to the reduced hydrodynamic heterogeneity.

Three contributions are dedicated to aspects of *process development and optimization*. Müller et al. [5] studied seed train intensification using an ultra-high cell density cell banking process. A frequently used approach for seed train intensification uses $N-1$ perfusion, in which perfusion cultivation is carried out as the final step of inoculum production to generate ultra-high cell banks exceeding 100×10^6 cells mL^{-1}. These cells can subsequently be used to inoculate a production bioreactor. On the one hand, the inoculum production steps can be reduced, and on the other hand a continuous process or a high-seed fed-batch process can be directly implemented with these cells instead of the otherwise usual low-seed fed-batch process. Within the study, an ultra-high cell density working cell bank was established for an immunoglobulin G-producing CHO cell line. A comparison with the standard approach shows that cell growth and antibody production are comparable, but time savings of greater than 35% are possible for inoculum production.

Ladd et al. [6] developed a process for continuous transfection for adeno-associated virus production in microcarrier-based culture. Adeno-associated virus vectors have great

potential for gene therapy. However, a major bottleneck for this kind of therapy is the limitation of production capacity. Higher specific AAV vector yield is often reported for adherent cell systems compared to cells in suspension, and a microcarrier-based culture is well established for the culture of anchored cells on a larger scale. The purpose of the present study was to explore how microcarrier cultures could provide a solution for the production of adeno-associated virus vectors based on the triple plasmid transfection of HEK293T cells in a continuously operated stirred tank bioreactor. The present investigation provided a proof-of-concept of a continuous process based on microcarriers in a stirred-tank bioreactor.

Petry and Salzig [7] developed a large-scale production process of size-adjusted β-cell spheroids. The large and growing number of patients living with diabetes has generated interest in the promise of β-cell therapy to restore lost β-cell mass. For β-cell replacement therapies, one challenge is the manufacturing of a sufficient number of functional β-cells manufactured as 3D constructs, known as spheroids with a controlled size. For this, a process in a fully controlled stirred bioreactor systems was established using the INS-1 β-cell line as a model for process development. Specifically, the dynamic agglomeration of β-cells to determine minimal seeding densities, spheroid strength, and the influence of turbulent shear stress was investigated in order to generate spheroids of a defined size. The process developed in shaking flasks was successfully transferred to a stirred bioreactor, and it could be shown that functional β-cell spheroids sufficient for β-cell therapy applications can be generated.

Two contributions are dedicated to the aspects of *monitoring and control of bioprocesses*. Reyes et al. [8] provide a review on modern sensor tools and techniques for monitoring and control, addressing especial technological innovation directed towards online in situ continuous monitoring of quality attributes that could previously only be estimated offline. These new sensing technologies when coupled with software models have shown promise for unique fingerprinting, smart process control, outcome improvement, and prediction. State-of-the-art sensing technologies and their applications in the context of cell culture monitoring are reviewed with an emphasis on the coming push towards industry 4.0 and smart manufacturing within the biopharmaceutical sector. Additionally, perspectives concerning how this can be leveraged to improve both understanding and outcomes of cell culture processes are discussed.

A new and promising biosensor technology is introduced by Gaudreault et al. [9], a surface plasmon resonance (SPR)-based biosensor. SPR-based biosensors can play a role in enabling the development of improved bioprocess monitoring and control strategies. In this review, the applications of SPR that are or could be related to bioprocess monitoring in three spheres are examined such as biotherapeutics production monitoring, vaccine monitoring, and bacteria and contaminant detection. These applications mainly exploit SPR's ability to measure solution species concentrations, but performing kinetic analyses is also possible and could prove useful for product quality assessments. SPR-based biosensors exhibit potential as product monitoring tools from early production to the end of downstream processing, paving the way for more efficient production control. However, more work needs to be carried out to facilitate or eliminate the need for sample pre-processing and to optimize the experimental protocols.

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References

1. Bayer, B.; Dalmau, D.R.; Melcher, M.; Striedner, G.; Duerkop, M. Digital Twin Application for Model-Based DoE to Rapidly Identify Ideal Process Conditions for Space-Time Yield Optimization. *Processes* **2021**, *9*, 1109. [CrossRef]
2. Rodríguez, T.H.; Sekulic, A.; Lange-Hegermann, M.; Frahm, B. Designing Robust Biotechnological Processes Regarding Variabilities Using Multi-Objective Optimization Applied to a Biopharmaceutical Seed Train Design. *Processes* **2022**, *10*, 883. [CrossRef]
3. Rodríguez, T.H.; Morerod, S.; Pörtner, R.; Wurm, F.M.; Frahm, B. Considerations of the Impacts of Cell-Specific Growth and Production Rate on Clone Selection—A Simulation Study. *Processes* **2021**, *9*, 964. [CrossRef]
4. Freiberger, F.; Budde, J.; Ateş, E.; Schlüter, M.; Pörtner, R.; Möller, J. New Insights from Locally Resolved Hydrodynamics in Stirred Cell Culture Reactors. *Processes* **2022**, *10*, 107. [CrossRef]
5. Müller, J.; Ott, V.; Eibl, D.; Eibl, R. Seed Train Intensification Using an Ultra-High Cell Density Cell Banking Process. *Processes* **2022**, *10*, 911. [CrossRef]
6. Ladd, B.; Bowes, K.; Lundgren, M.; Gräslund, T.; Chotteau, V. Proof-of-Concept of Continuous Transfection for Adeno-Associated Virus Production in Microcarrier-Based Culture. *Processes* **2022**, *10*, 515. [CrossRef]
7. Petry, F.; Salzig, D. Large-Scale Production of Size-Adjusted β-Cell Spheroids in a Fully Controlled Stirred-Tank Reactor. *Processes* **2022**, *10*, 861. [CrossRef]
8. Reyes, S.; Durocher, Y.; Pham, P.L.; Henry, O. Modern Sensor Tools and Techniques for Monitoring, Controlling, and Improving Cell Culture Processes. *Processes* **2022**, *10*, 189. [CrossRef]
9. Gaudreault, J.; Forest-Nault, C.; Crescenzo, G.; Durocher, Y.; Henry, O. On the Use of Surface Plasmon Resonance-Based Biosensors for Advanced Bioprocess Monitoring. *Processes* **2021**, *9*, 1996. [CrossRef]