THE CRITICAL ROLE OF PREDICTIVE BIOREACTOR CHARACTERIZATION IN PHARMACEUTICAL PROCESS-BASED UPSCALING

In bioreactors, microorganisms or cell cultures produce complex therapeutic proteins and other biopharmaceuticals. The industrial production of those active pharmaceutical ingredients usually involves a seed train: the cells are run through many cultivation systems, which become larger with each passage (Upstream Process). An adequate number of cells for the inoculation of large-scale production bioreactors of 10,000 liters or more is generated.

A prominent example from the growing mammalian cell culture processing sector is the upstream production process of monoclonal antibodies. These are very specific and complex proteins used for cancer therapy and, recently, for treating severe Covid19 infections. The European Commission approved the SARS-Cov-2 neutralizing antibody Sotrovimab by GlaxoSmithKline in December 2021. Recombinant DNA technology produces monoclonal antibodies in Chinese Hamster Ovary (CHO) cells. [1,2]

The CHO cell is a genetically modified living organism. Its proliferation – and, in further consequence, the quality and yield of the final product - depends on the growth conditions in the bioreactor system. Nowadays, there is a profound understanding of environmental conditions (nutrient supply, pH, temperature, mechanical cell damage) on cell growth and product formation.

To achieve the optimum conditions, the design of the bioreactor and a sophisticated selection of parameters within the fermentation process is crucial. Variations in that respect are expected to have a significant impact on production.

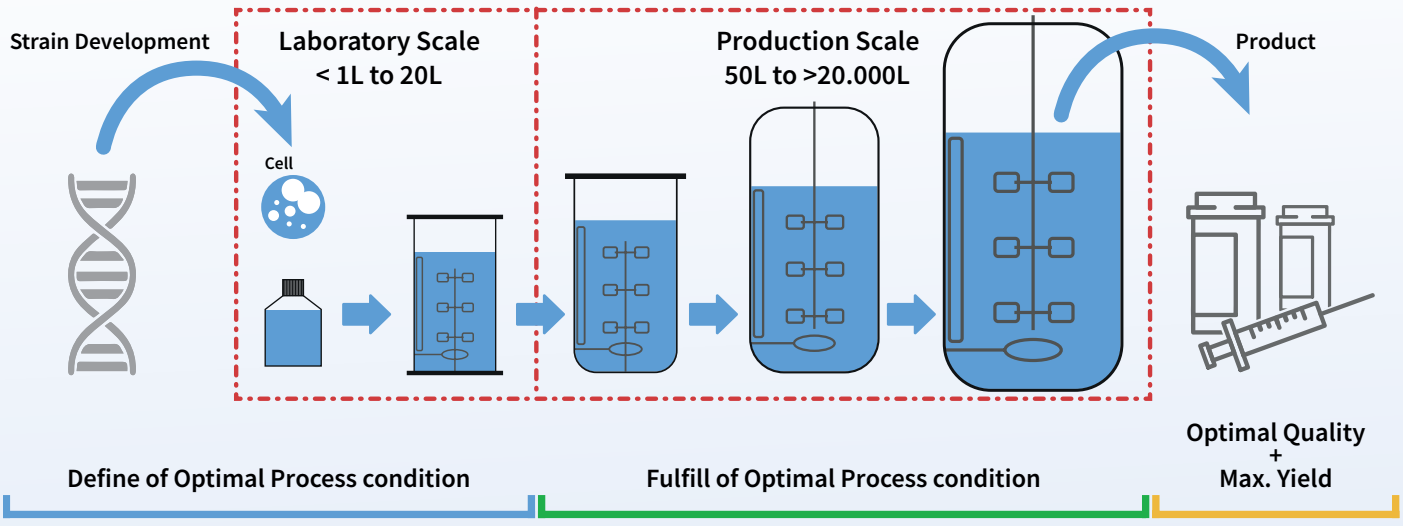

Figure 1: Scaling for biopharmaceutical production: from process development via production process (seed train) to the final product

Naturally, scale-up activities entail variations, and when transferring a biopharmaceutical process from laboratory to production plant scale, the impact of these changes must be given thorough consideration. Scaling in a cell culture upstream process, including the typical seed train, is depicted in Figure 1.

For the validation of a new process, the FDA, therefore, clearly defines:

"Manufacturers should:

- Understand the sources of variation.
- Detect the presence and degree of variation.
- Understand the impact of variation on the process and ultimately on product attributes.
- Control the variation in a manner commensurate with the risk it represents to the process and product." [3]

Biopharmaceutical products are developed and validated in the lab. The operating parameters are well defined, and the small-scale equipment used for product formation is well characterized. Nevertheless, when it comes to large-scale production systems of more than 1,000 liters, available information on operating parameters and system characteristics is somewhat scarce.

The goal in the design of the bioreactors used for industrial upstream processing is to maintain similar process conditions to those which apply on a small scale during product development and validation. This raises the question: "to what extent is the replication of the environmental conditions of a small-scale lab reactor applicable to that of a large-scale bioreactor of 20,000 liters or more?" To fully answer this question, it is necessary to define the critical process parameters which impact product yield, cell growth, and quality, and can be reliably determined.

Before doing so, the current procedure for transferring a bioreactor design and operating parameters from laboratory to production scale needs to be described.

Scaling of bioreactors

Standard scaling approach

In bioreactor scaling, two important aspects are to be considered: (1) the design and (2) the operating parameters. For both aspects, numerous guidelines are available.

The most common practice in terms of design is to maintain the ratio between the height of the liquid level and the inner diameter (H/D value) and keep as many other geometrical ratios the same while setting the overall dimensions. The same concept is applied to the geometry of the equipment inside the bioreactor, like agitator, baffles, and sparger. When it comes to operating parameters, the approach for upscaling is volume-based. The volumetric power input (kW/m^3) and the volumetric gassing rate (vvm= Vessel Volume per minute) are calculated and kept constant. This entire concept is based on the assumption that a system with similar geometries and a similar operation setup will provide the same environmental

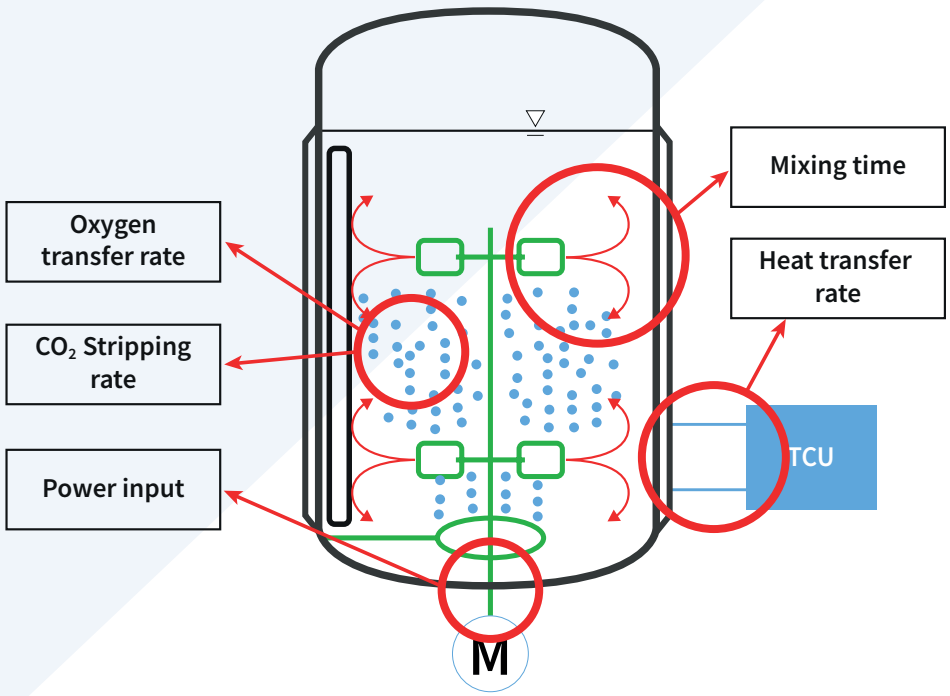

Figure 2: The key performance characteristics of a bioreactor and their determination

conditions for cell growth, even when the operating volume is significantly increased. As all production critical process parameters, like mixing time, heat transfer rate, and oxygen transfer rate, result from these selections, this assumption-based traditional approach should be challenged to achieve alignment with the FDA's stringent requirements for process transfer.

Bioprocess-based scaling approaches for reliable process transfer

By focusing on the process conditions that ensure an optimal environment for cell cultivation, and taking key performance indicators as the basis for the design of the bioreactor and its equipment, new scaling approaches are conceivable. In a possible process-based scaling scenario, essential process conditions, like oxygen transfer rate, mixing time, heat transfer, or CO2 stripping rate, are defined and prioritized according to the needs of the respective organism. The changeable variables for the adjustment of the defined process conditions are the equipment design parameters (vessel design, agitator design, sparger design) and the operating parameters (agitator speed, gassing rate, temperature).

Characterization of industrial-scale bioreactors

The key to the application of a process-based scaling concept lies in understanding the impacts that variations of certain parameters have on product quality and yield. The calculation and modeling of performance indicators for bioreactors, like the kLa value and mixing time, is complex and still inaccurate. Thorough bioreactor characterization leads to insights beneficial for scaling and makes it easier to prove regulatory requirements.

Any parameter that can be measured in a bioreactor constitutes a distinctive advantage for a better understanding of the process. [4] In terms of process-based scaling of bioreactor systems, reliable measuring methods with comparable results are essential to ensure that the process parameters remain within a defined design space and that the optimal conditions are fulfilled.

Certain constraints need to be considered when it comes to industrial bioreactors and their design, optimization, and characterization. These involve mechanical changes, the usage of approved media and components, and general restrictions that apply at pharmaceutical plants, especially in clean rooms. A huge variety of measuring methods for process parameters in bioreactors are available, but most of them were developed for laboratory equipment.

Measurable parameters of industrial-scale bioreactors

The prediction of the key performance characteristics of a bioreactor (see Figure 2) is difficult or even impossible, and they depend on numerous intricately interrelated factors. It is critical to determine them by methods based on reliable measurements. ZETA has developed advanced methodologies for the assessment of several important performance parameters and executes bioreactor characterizations based on these measurements and calculations.

Heat transfer rates. Metabolic processes in living organisms produce heat. This is especially relevant for bacterial fermentation, as bacteria produce large amounts of heat that must be removed from the system to prevent cell damage. Precise

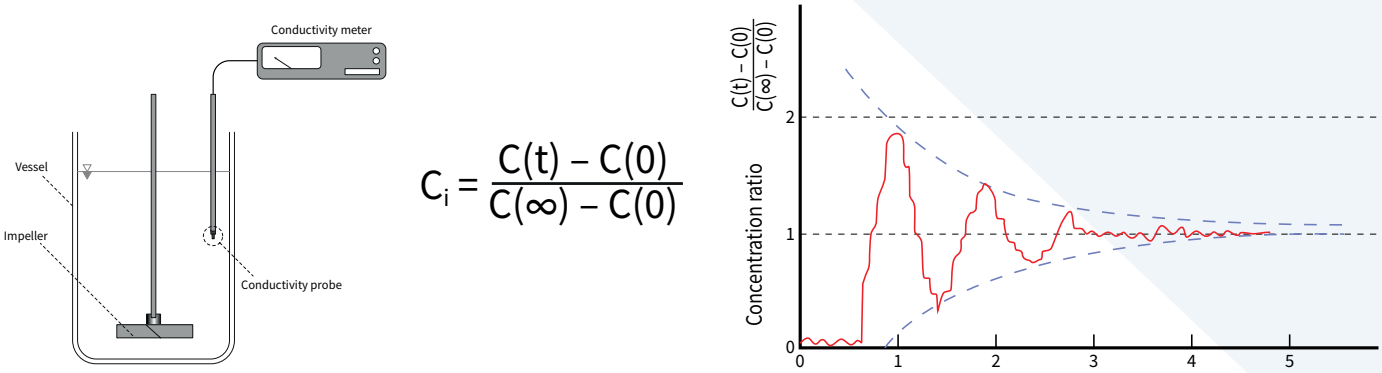

Figure 3: Determination of mixing time by conductivity method. The tracer concentration is followed over time. The mixing time is the time to achieve a predefined level of homogeneity - e.g., ±5% deviation of the final concentration (or conductivity). C…conductivity [S m-1], C(t)… conductivity at a certain time t, C(0)…initial conductivity, C(∞)…final conductivity; Source: G. Ascanio, Chinese Journal of Chemical Engineering Mixing time in stirred vessels : A review of experimental techniques, CJCHE. 23 (2015) 1065–1076. doi:10.1016/j.cjche.2014.10.022.

temperature control is essential to maintain optimal process conditions. The determination of heat transfer rates is straight-forward and should be included in the commissioning procedure of a new bioreactor system without complications.

ZETA evaluates the heat transfer rate by indicating a temperature change using the temperature control unit and measuring the resulting heat balance. The inlet temperature, outlet temperature, and mass flow rates are measured by using a flow meter, a set of temperature probes, and a data logger.

Further relevant parameters are then calculated. Calculation tools support the prediction of heat-up and cooling times and the design of heat exchangers. Furthermore, the information gained can be used for the evaluation of required cooling water temperatures or when internal cooling coils are required for a bioreactor design.

Mixing time. In a stirred vessel, mixing time is a key parameter for performance analysis. Efficient mixing is essential for the distribution of nutrients and for a quick response to changes in process conditions, like pH and foaming. The effect of the agitator speed on the nutrient distribution inside the bioreactor is evaluated by determining the mixing time. Furthermore, the mixing time has to be taken into account during the addition of medium components (for example, during the adjustment of pH) to avoid overdosing.

Mixing time is defined as the time to achieve a predefined level of homogeneity of a tracer in a stirred vessel and can be determined experimentally (see Figure 3) or through numerical modeling, like computational fluid dynamics (CFD). The experimental methods include a conductivity method and a colorimetric method. The former requires a conductivity probe inside the bioreactor system. Saturated salt water is added to the system, and the level of conductivity is monitored over time with the sensor and a data logger. The colorimetric method uses a colored indicator as a tracer. [5] The colorimetric method is carried out in a glass vessel using a pH indicator and shifting the pH value with acid and base.

Power input. Another parameter that is essential for the characterization of the agitator in a stirred tank is the power input. The power the agitator transmits to the system results in liquid movement but also shear stress and friction. Finding the appropriate balance between efficient mixing and decent gas distribution while avoiding physical cell stress is crucial. The calculation of an agitator's power input is based on its power number, Np. It indicates what power is required to operate the agitator at a certain speed. The power number is specific to the impeller geometry and to the system where the agitator is installed (e.g., baffles in the tank, vessel-to-stirrer diameter ratio, fill level, gassing rate, etc.), but not to the size of the system. Therefore, Np is of major importance for scale-up procedures. Theoretically, Np can be transferred to any scale of the system. In practice, this only leads to reliable results in a limited range of sizes, as not all the geometric relationships of a stirrer system that prevail on a relatively small scale are transferred one-to-one to a very large scale.

$$N_p = \frac{P}{\rho\, n^3 d_R^5}$$

$$Re = \frac{\rho\, n\, d_R^{\,2}}{\eta}$$

P [W]
ρ [kg/m²]
n [1/s]
d_R [m]
η [kg/m s]

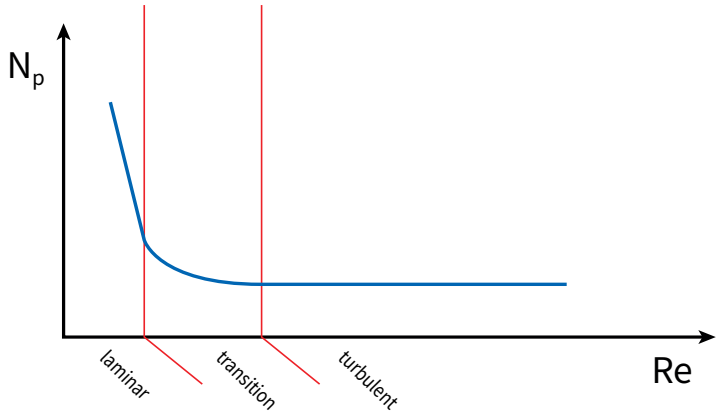

Figure 4: Schematic illustration of the performance characteristics of an agitator. The power number Np contains all the information on the resistance and friction forces of the agitator. The power number depends on the Reynolds number Ne and, therefore, the liquid properties and stirring speed for the design of individual agitators. P… power [W], ρ… density [kg/m3], n… rotational speed [s-1)], dR… agitator diameter [m], η… dynamic viscosity [Pa s]

The power number contains information on the resistance and friction forces of a specific agitator. The power number of a specific agitator is correlated to the Reynolds number (Re), which depends on the properties of the liquid (density, viscosity) and the stirring speed. (For a schematic illustration of the performance characteristics of an agitator, see Figure 4). To calculate the actual power number of a stirrer system, the power input of the agitator is determined. The adequate measuring device available at ZETA is a torque sensor with a connection flange, which is installed between the motor and the driving magnet.

Oxygen uptake rate and k_La. AAerobic microorganisms can only metabolize oxygen that is dissolved in their cultivation media. Maintaining a decent oxygen concentration during cultivation is therefore critical to guarantee cell growth. Ideally, the oxygen transfer rate (OTR) into the system is equal to the oxygen uptake rate of the cells. The OTR is a function of the volumetric mass transfer coefficient (kLa), one of the key parameters for bioreactor design. [6,7] Their calculation and modeling are extremely complex, as they are influenced by agitation, aeration rate, and numerous other geometrical and physical factors (see Figure 5). A variety of methods for the determination of the kLa value are available. The most commonly used and recommended by the DECHEMA (expert network for chemical engineering and biotechnology in Germany) is the dynamic step method. Here the bioreactor is filled with purified water, and nitrogen is used to strip the oxygen from the system. Then the system is gassed under defined conditions using air, and the saturation process is monitored. The kLa value is then calculated from this dynamic change.

ZETA's approach for optimized up-scaling based on process conditions

ZETA designs and constructs customized bioreactors for a huge variety of processes and products. Characterization of each bioreactor is performed by ZETA as standard procedure. The result is an ample and further growing database for vessels with working volumes from 20 L to 20,000 L with numerous geometries and operating points.

Following the standard scaling approach, and with the incorporation of valuable information from this database, models are generated to predict the key performance parameters of new bioreactor designs. These predicted values are then evaluated. Do they comply with the values set in process development that are expected for optimal cell growth? If

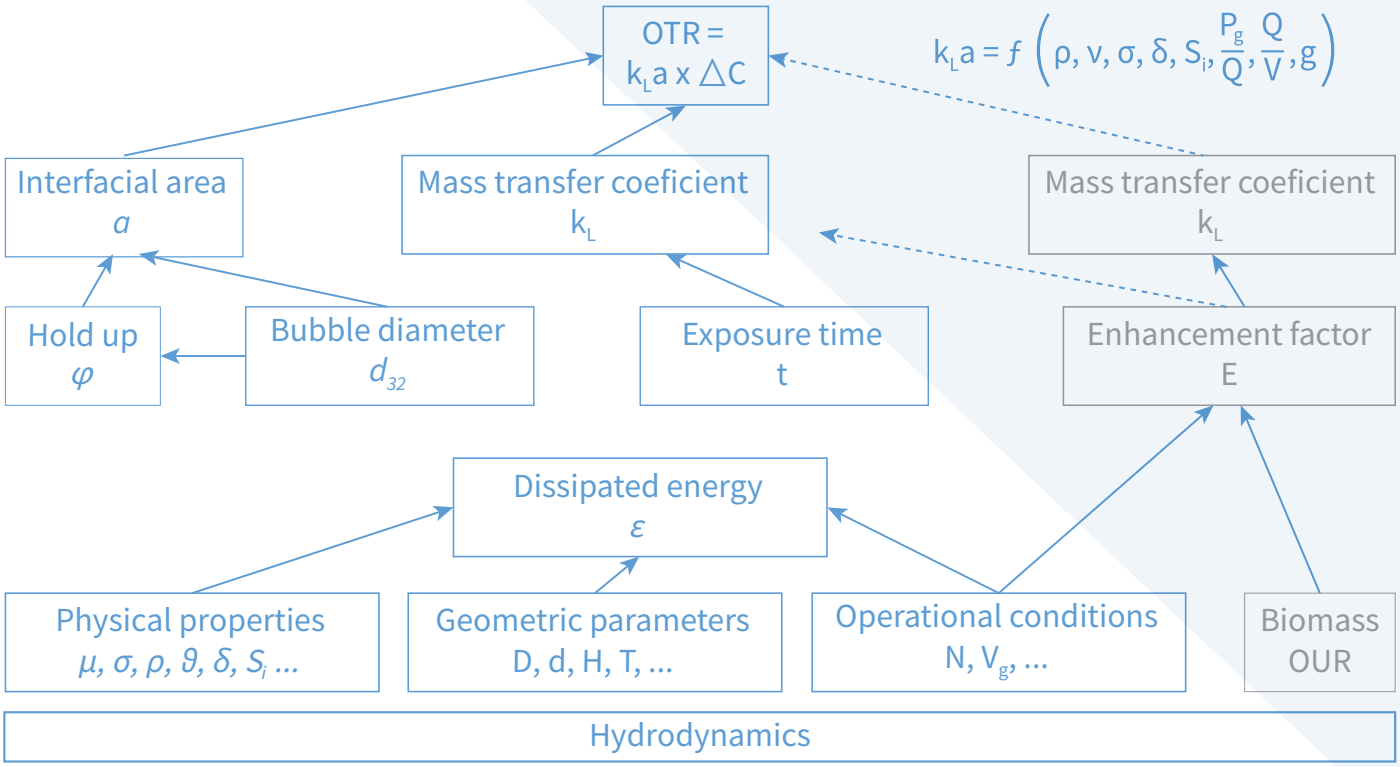

Figure 5: The oxygen transfer rate is influenced by numerous factors. Source: Maischberger, T.; Optimized Process and Bioreactor Characterization; Chem.Ing.Tech. 2019, 91, No.12, 1719-1723

the predicted performance parameters differ from these specific requirements, an optimized alternative of the system is modeled. The turning screws are the vessel geometry, the design of the agitator, and the operating parameters. Based on that model, a scale-up strategy is developed with the aim to provide optimal process conditions that lead to maximum yield and quality. The resulting model can also be scaled back to lab scale (see Figure 6).

Conclusions

The scale-up strategies currently used in the biopharmaceutical industry are founded on the premise that simple geometrical scaling for the industrial bioreactor will sufficiently resemble the conditions set during product development at lab scale. It is time to bring this assumption into question to optimize the existing processes. Reliable and sound data must be drawn upon when the current scale-up strategies are examined or new methods are developed. A highly valuable data source is the characterizations of bioreactor systems by proven methodologies.

In an innovative, process-based approach, the scale-up of bioreactor systems is based on the specific process conditions, described by performance parameters including the oxygen transfer rate, power input, mixing time, or the heat transfer rate. Having defined measuring procedures for the essential parameters, ZETA assimilates a large volume of valuable data and leverages its specific knowledge that results from their ample experience in bioreactor design, characterization, and optimization. New scaling strategies are developed based on standard scaling procedures and a growing database of measured process parameters.

The scale-up approach proposed by ZETA involves a deep understanding of the processes and clear definitions of critical process parameters. These parameters can then be challenged and adhered to in the bioreactor design and, most importantly, verified by characterization during factory and site acceptance tests (FAT and SAT). This optimized approach guarantees the maximum product quality and yield and compliance with the FDA guidelines for process validation.

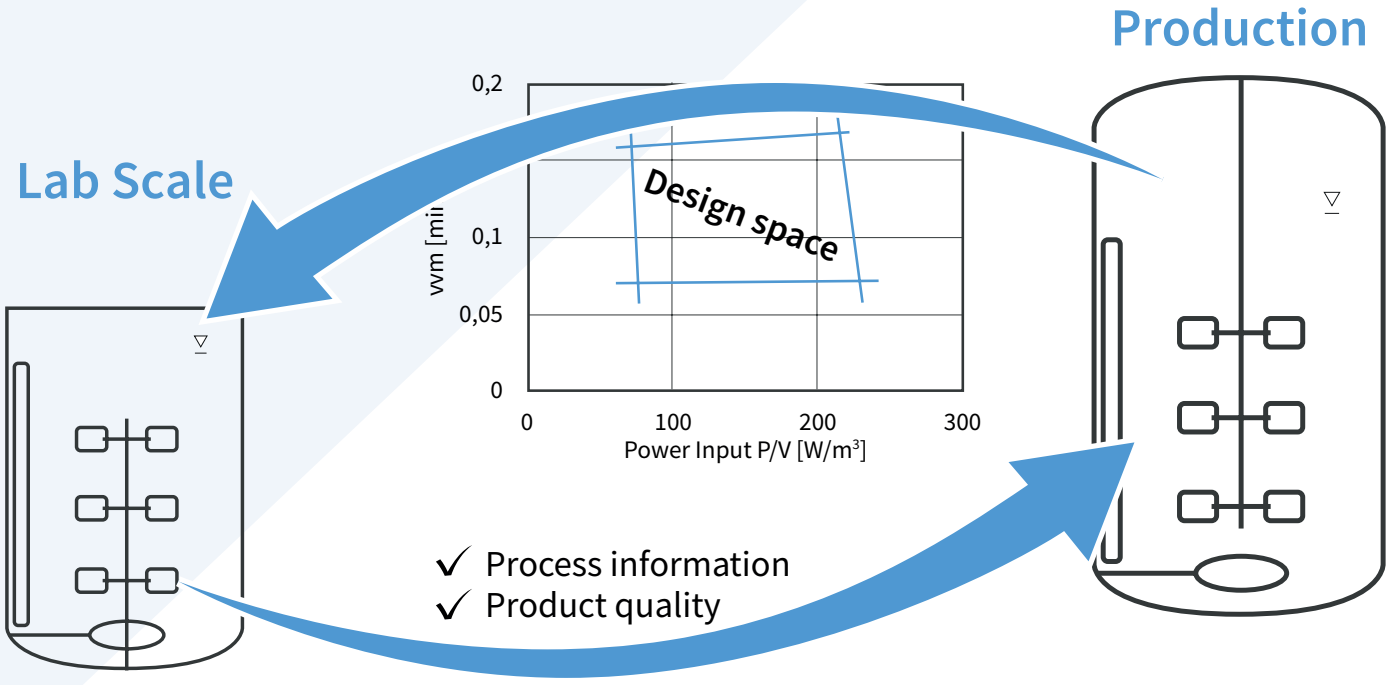

Figure 6: Scale-up strategy based on an advanced prediction model and experimental verification by scaling back to lab scale.

References

[1] https://www.pei.de/DE/newsroom/hp-meldungen/2021/211217-eu-zulassung-antikoerper-xevudy-gegen-covid-19.html

[2] https://www.medicines.org.uk/emc/product/13097/smpc#gref

[3] US Department of Health and Human Services, FDA, CDER, CBER, CVM; Guidance for Industry Process Validation: General Principles and Practices; January 2011

[4] Maischberger, T.; Optimized Process and Bioreactor Characterization; Chem.Ing.Tech. 2019, 91, No.12, 1719-1723

[5] Bai, G., Armenante, P. M., Plank, R. V., "Experimental and Computational Determination of Blend Time in USP Dissolution Testing Apparatus II," Journal of Pharmaceutical Sciences, Volume 96, Issue 11, Pages 3072-3086, 2007

[6] D. Moutafchieva, D. Popova, M. Dimitrova, S. Tchaoushev, Experimental Determination of the volumetric mass transfer coefficient, J. Chem. Technol. Metall. 2013, 48 (4), 351–356.

[7] A. Karimi et al., Oxygen mass transfer in a stirred tank bioreactor using different impeller configurations for environmental purposes, Iran. J. Environ. Health Sci. Eng. 2013, 10 (1), 6.DOI: https://doi.org/10.1186/1735-2746-10-6

CONTACT

SCAN AND LEARN MORE
www.zeta.com

Florian KRAINER
Process Engineer & Project Development
Project Development

ZETA GmbH
+43 (3136) 9010-1354
florian.krainer@zeta.com

ABOUT ZETA

The ZETA Group, with 1000 highly qualified employees and 17 subsidiaries worldwide, specializes in the design, manufacturing, automation, digitalization and qualification of customized biopharmaceutical facilities for aseptic process solutions. ZETA acts as a one-stop shop, combining plant engineering with HVAC, cleanroom and BMS/EMS design.

Biopharmaceutical active ingredients, such as anti-cancer drugs, insulin, vaccines and infusions are produced in these highly complex, "tailor-made" facilities. ZETA supports its customers along the entire drug development and manufacturing pathway with sophisticated solutions from laboratory to industrial production scale. Through its Smart Engineering Services, ZETA creates digital twins of process plants and has thus established itself as a market leader for digital solutions in the pharmaceutical and biotech industry.