

# Scale-Down Model Development in ambr systems: An Industrial Perspective

Viktor Sandner,\* Leon P. Pybus, Graham McCreath, and Jarka Glassey

High-Throughput (HT) technologies such as miniature bioreactors (MBRs) are increasingly employed within the biopharmaceutical manufacturing industry. Traditionally, these technologies have been utilized for discrete screening approaches during pre-clinical development (e.g., cell line selection and process optimization). However, increasing interest is focused towards their use during late clinical phase process characterization studies as a scale-down model (SDM) of the cGMP manufacturing process. In this review, the authors describe a systematic approach toward SDM development in one of the most widely adopted MBRs, the ambr 15 and 250 mL (Sartorius Stedim Biotech) systems. Recent efforts have shown promise in qualifying ambr systems as SDMs to support more efficient, robust and safe biomanufacturing processes. The authors suggest that combinatorial improvements in process understanding (matching of mass transfer and cellular stress between scales through computational fluid dynamics and in vitro analysis), experimental design (advanced risk assessment and statistical design of experiments), and data analysis (combining uni- and multi-variate techniques) will ultimately yield ambr SDMs applicable for future regulatory submissions.

## 1. Introduction

The biopharmaceutical industry faces an increasing demand to accelerate the timeline to develop commercial cell culture processes. A key regulatory requirement is to demonstrate management of product quality control through the identification of critical quality attributes (CQAs) and their controlling critical process parameters (CPPs). Such process characterization

requires the understanding of a multidimensional design space in which multiple CPPs can impact CQAs.<sup>[1]</sup> It is therefore unfeasible to conduct such studies at manufacturing scale and representative scale-down models (SDMs) must be utilized to adequately interrogate the design space. Traditionally, bench-scale bioreactors (BSBs; 1 to 10 L scale) are utilized as upstream SDMs,<sup>[2–8]</sup> however, increasing throughput requires the implementation of high-throughput (HT) technologies.

HT small-scale culture systems such as miniature bioreactors (MBRs), have been widely adopted within the industry and are increasingly utilized throughout the lifecycle of a biopharmaceutical drug product. Such systems were initially utilized as screening tools (e.g., cell line selection and process optimization during early-phase clinical development,<sup>[9–16]</sup> but increasing interest is focused towards their use as SDMs of the cGMP manufacturing scale processes during late-phase clinical development.<sup>[17–21]</sup> These

systems offer the potential to shift the bottleneck away from the resource limitations associated with BSBs. However, the application of HT technologies shifts the bottleneck to sample and data analysis, which becomes rate limiting, unless effective workflows are in place.

Several competing MBR technologies have been developed, including BioProcessors' SimCell microbioreactors,<sup>[22]</sup> microtiter plates,<sup>[23]</sup> Pall's micro24,<sup>[24]</sup> shake flasks,<sup>[25]</sup> and spin tubes.<sup>[26,27]</sup> However, the micro- and mini-scale bioreactor systems ambr15 (10–15 mL working volume) and ambr250 (100–250 mL working volume) have largely become industry standard MBRs.<sup>[9–21]</sup>

The focus of this study is to evaluate the new challenges faced when using ambr systems as qualified SDMs. One of the very first questions to answer is which SDM criterion to select and this is not a trivial answer for a MBR that is geometrically dissimilar to its larger scale counterpart. However, the HT data generating capability of ambr systems can be leveraged to identify the correct SDM criteria for a given cell line/process. Therefore, experimental design, data management, and data analysis must all be carefully considered. With such a *modus operandi* we can look forward to ambr systems providing SDM data for future regulatory submissions.


## 2. The Importance of SDM Criteria

During the development of a SDM it is important to demonstrate general process performance and equivalency of CQAs between

Dr. V. Sandner, Dr. G. McCreath  
Process Design, Process Development  
FUJIFILM Diosynth Biotechnologies  
Belasis Avenue, Billingham, TS23 1LH, United Kingdom  
E-mail: viktor.sandner@fujifilm.com

Dr. V. Sandner, Prof. J. Glassey  
School Engineering  
Merz Court University of Newcastle  
Newcastle Upon Tyne, NE1 7RU, United Kingdom

Dr. L. P. Pybus  
Mammalian Cell Culture, Process Development  
FUJIFILM Diosynth Biotechnologies  
Belasis Avenue, Billingham, TS23 1LH, United Kingdom

 The ORCID identification number(s) for the author(s) of this article can be found under <https://doi.org/10.1002/biot.201700766>.

DOI: 10.1002/biot.201700766

small-scale and commercial manufacturing scale. Industrial users of the ambr system including Biogen,<sup>[19]</sup> BMS,<sup>[21]</sup> Genentech,<sup>[12]</sup> KBI,<sup>[20]</sup> Merck,<sup>[10,16,17]</sup> MedImmune,<sup>[15]</sup> and UCB<sup>[11,18]</sup> have published their observations comparing CQAs between ambr systems and BSBs. A brief overview of selected important events in the ambr timeline can be followed in Figure 1.

However, ideally cell culture performance at commercial manufacturing scale should be used as a baseline to guide SDM development and few examples exist that compare CQAs in the ambr with manufacturing scale.<sup>[17–21]</sup> Nevertheless, equivalence to bench-scale performance does still provide encouraging baseline data to provide preliminary information for SDM development.

A good SDM needs to not only match commercial manufacturing scale performance but also the response of changing process parameters, which can be categorized into scale-dependent (e.g., working volume, feed volume, agitation, aeration) and scale-independent parameters (e.g., pH, dissolved O<sub>2</sub>/CO<sub>2</sub>, temperature, media/feed composition, inoculation ratios, and feed regimes). A general strategy for SDM development is to proportionally scale down the scale-dependent parameters whilst maintaining the scale-independent parameters at the same set-points used in the manufacturing scale process. However, difficulties can occur during the linear scale-down of scale-dependent and the matching of scale-independent parameters due to differences in bioreactor geometry, liquid surface-to-volume ratio, gassing regime, and control capability in ambr systems.

Unfortunately, the aforementioned industrial publications on SDM development in ambr systems do not always detail their SDM criteria (with a few exceptions, which are not always peer-reviewed – see Notes and Supporting Information. The Supporting Information contains a table with typical operational setpoints for ambr systems in mammalian cell culture and a few notes on the experiments). However, available information and points to consider when choosing SDM criteria for ambr systems are detailed below.

## 2.1. Agitation and Aeration Considerations in the ambr15

One of the most important SDM criteria is the maintenance of mass transfer (O<sub>2</sub> supply, CO<sub>2</sub> stripping and bulk mixing) by agitation and gas sparging. Traditional SDM criteria include matching power per unit volume ( $P/V$ ), tip speed ( $ts$ ), impeller shear rate ( $\gamma$ ), specific impeller pumping rate ( $Q_s$ ), gas flow rate per volume (volume of gas per vessel volume per minute;  $vvm$ ), volumetric mass transfer coefficient ( $kLa$ )/oxygen transfer rate (OTR), and CO<sub>2</sub> stripping rate.<sup>[28–30]</sup>

Although a limit to gas sparging and agitation exists due to the shear sensitivity of CHO cells, it is no longer considered to be a major problem due to the addition of surfactants such as Pluronic-F68 to media formulations.<sup>[31,32]</sup> Therefore, today critical SDM criteria include oxygen transfer, bulk liquid mixing, and dCO<sub>2</sub> removal.<sup>[28–30,33]</sup>

It is perhaps unsurprising that the first publications on SDM development in ambr systems have largely focused on scaling based on traditional engineering parameters, such as setting agitation speed through  $P/V$  or  $ts$  rather than through detailed



**Viktor Sandner** is a Data Scientist at Fujifilm Diosynth Biotechnologies, where he supports agile cross-department (CLD, USP, AD, DSP, and QC) digitization activities to track, connect, visualize and analyse big data. He developed statistical and CFD models to help quantify equivalence during scale up/down and tech transfer, which can support

regulatory submission. Viktor enjoys communicating his results to the CSO Level as well as to the general public and teaches how to get started with machine learning. He holds a PhD in Chemical Engineering from Technical University Vienna.



**Leon Pybus** is Staff Scientist within the Mammalian Cell Culture R&D group at FUJIFILM Diosynth Biotechnologies. Here he has helped to develop both the Apollo(TM) mammalian expression system and upstream Saturn(TM) mAb platform. He holds a PhD from the University of Sheffield in Chemical and Biological Engineering.

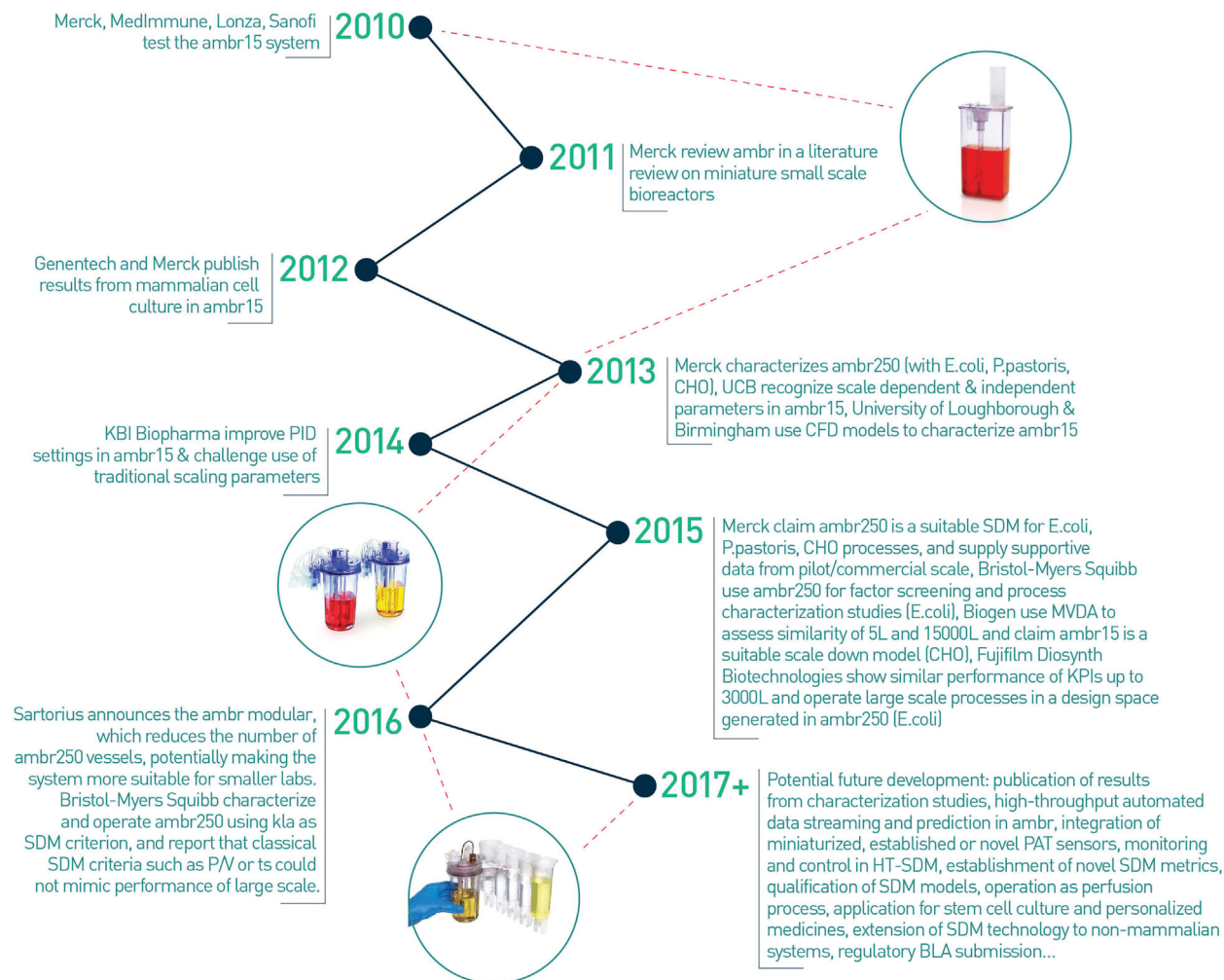


**Graham McCreath** leads a Late Stage CMC and Data Science Group in Process Development at FUJIFILM Diosynth Biotechnologies. Graham has been intimately involved in a number of product commercial approvals at Fujifilm and as such has had numerous interactions with Global Health Authorities such as EMA and FDA.

Graham has on-going interests in how Data Science can help reconcile, focus, exemplify and demonstrate biologics manufacturing process control. He holds a PhD in Chemical Engineering from the University of Cambridge.

experimental characterization of oxygen transfer, bulk liquid mixing, and dCO<sub>2</sub> removal.

One of the first peer-reviewed industrial studies of the ambr15 was Genentech's evaluation of the ambr15 as a scale-down mimic of their 2 L BSB.<sup>[12]</sup> Scaling based on  $P/V$  and equivalent  $vvm$  air sparging rate resulted in the most comparable DO profiles and culture performance. However, dO<sub>2</sub> profiles did take longer to reach the 30% set-point in the ambr15 and spikes occurred in the ambr15 dO<sub>2</sub> profiles, corresponding to additions of antifoam/feed and during liquid addition/sampling when O<sub>2</sub> is being sparged into the vessels as they disturb the head space and alter the working volume. Such variation in dO<sub>2</sub> may not be a concern for CHO cells which are generally considered insensitive to dO<sub>2</sub> levels between 10 and



**Figure 1.** The ambr timeline. A short overview of recent publications using ambr systems and potential future development around HTPD in SDM.

80%.<sup>[34,35]</sup> The increased time to reach the  $dO_2$  set-point may be explained by the observation that agitation rate exerts a higher degree of control over  $k_La$  in ambr15 in comparison to larger scale bioreactors.<sup>[36]</sup>

This early effort to scale up or down based on  $P/V$ <sup>[12]</sup> may have underestimated the required agitation rate for matched  $P/V$ . Today's industrial processes have much higher cell concentrations resulting in too low operational RPM to hold  $dO_2$ , as we have experienced with some of our newer cell lines. The  $P/V$  for an ambr15 vessel at a given  $ts$  is 10–12 fold higher than conventional bioreactors, which is a direct consequence of the different physical characteristics of the ambr15 and makes it particularly unsuitable for this system.<sup>[36]</sup>

Thus, when considering SDM criteria for the ambr15 system,  $P/V$  should not be chosen to determine the agitation rate for modern cell culture processes. It is important to note that  $ts$  scaling in ambr15 systems usually utilizes BSBs at the comparator and this SDM criteria may not be appropriate for all bioreactor scales as either the scaled values are not practical or  $ts$  is not the limiting parameter,<sup>[32,37,38]</sup> indicating that better SDM metrics may have to be established.

In an article published in 2012 by Hsu at Merck, agitation rates were scaled between the ambr15 (900 rpm) and a 3-L BSB (200 rpm) using a  $ts$  of  $0.5 \text{ ms}^{-1}$  and a  $dO_2$  set point of 60%.<sup>[16]</sup> Utilization of a lower agitation speed and higher  $dO_2$  set point resulted in comparable  $dO_2$  profiles between the BSB and the ambr15 system. The performance of two mAb producing cell lines were evaluated in both systems as cell culture performance indicators (growth/viability, titer, and product quality) were similar in both the BSB and the ambr15.

In 2014, KBI published a study where the ambr15 was found to produce matched cell culture performance (growth/viability and titer) across scales (3, 15, and 200 L).<sup>[20]</sup> Importantly, the authors found that the ambr15 reproduced perturbations in pH,  $DO_2$  and temperature in a similar manner to larger scale systems. Agitation was set to 1000 rpm based on a matched  $ts$  between the ambr15 system and their 3 and 15 L BSBs. In agreement with the observations of Hsu et al.<sup>[12]</sup> relatively short (30–60 min) perturbations were observed in  $dO_2$  during bioreactor sampling and base/feed additions.

The SDM criteria initially adopted for the ambr15 by Biogen was that of matching  $ts$  with their 5 L BSB.<sup>[19]</sup>  $pCO_2$  profiles at

15 000 L manufacturing scale were replicated in the ambr15 through the introduction of a variable air cap and comparable titer, growth and product quality characteristics were obtained between scales. Furthermore, acceptable process parameter ranges were comparable both in the ambr15 and a 5 L BSB.

A physical characterization study by Nienow et al.<sup>[36]</sup> utilized a combination of experimental and computational methods to report power number,  $kLa$  and mixing time for ambr15 vessels. Whereas the flow regime in a 5-L BSB was found to be essentially turbulent, the flow regime in the ambr15 was found to be transitional. The authors hypothesized that matched culture performance across scales may be due to a balance between fluid mechanical stress (agitation and bursting bubbles) and environmental heterogeneity (nutrients, pH,  $dO_2$ ,  $dCO_2$ , or osmolality). Stress in the ambr15 may be focused more towards fluid mechanical stress and environmental heterogeneity may be more of a concern at increased scale.

Currently, decomposing the exact contribution of stresses to poor process performance/cell death with analytical tools is challenging. There is a lower priority to measure stress signals compared to established metabolic markers. Even if stresses are measured in subpopulations, the root cause for apoptosis, lysis or necrosis may not be entirely clear. A similar stress level should be established by carefully selecting appropriate parameters during scaling up/down, which adequately represent process performance at commercial scale.

## 2.2. Agitation and Aeration Considerations in the ambr250

ambr250 vessels are more geometrically similar to larger-scale systems than the ambr15. This theoretically enables key engineering assumptions (e.g., consistent power input) to be applied and result in maintenance of similar fluid dynamics and flow properties.

One of the first peer-reviewed industrial studies of the ambr250 was Merck's evaluation of the ambr250 as a SDM of a 3 L BSB and a 200 L bioreactor.  $P/V$  was utilized as a SDM criteria and two CHO cell lines were shown to reach comparable cell culture performance (cell growth/viability, titer).<sup>[10]</sup> Data on comparable mAb quality for one cell line was also reported. The same group also reported utilizing the same SDM criteria ( $P/V$ ) to reach comparable cell growth/viability, metabolic profiles, titer and product quality for a CHO cell line at ambr250, 3 L, 650 L, and 2500 L scales.<sup>[17]</sup> Interestingly,  $pCO_2$  profiles were shown to be different across the different scales utilized.

In 2017, BMS published a paper on SDM criteria selection in the ambr250.<sup>[21]</sup> In this study, SDM criteria including  $vvm$ ,  $P/V$ , and  $kLa$  were assessed using different CHO cell lines and cell culture performance between the ambr250, 5 L BSBs, 250 L, and 1000 L scales was evaluated. The ambr250 was found to require higher  $vvm$  flow rates to achieve the same  $kLa$  at matched  $P/V$  to larger scale systems. The authors hypothesized that this was due to the difference in sparger design between the ambr250 (open pipe) and either drilled hole or drilled hole and frit spargers utilized in larger scale systems.

The  $kLa$  values obtained ( $2\text{--}14\text{ h}^{-1}$ ) were comparable to those obtained by Bareither et al. ( $2.5\text{--}8.5\text{ h}^{-1}$ ).<sup>[10]</sup> The  $kLa$  values reported for the ambr15 are between  $2.1$  and  $12.97\text{ h}^{-1}$ <sup>[36]</sup> and the

$kLa$  values of the ambr250 are slightly lower than those reported for the ambr15 at the same  $vvm$ . When  $kLa$  was utilized by BMS as a SDM criteria for cell line A, ambr250 growth profiles and titer matched with those of 5 and 250 L bioreactors. For cell line B, the cell growth of the ambr250 matched those of 5, 250, and 1000 L bioreactors with 2 day delay in peak cell density and the titer of the ambr250 varied  $\approx 5\%$  from that obtained at larger scale. A further, six CHO cell lines were compared in the ambr250 and in a 5 L BSB. The cell growth characteristics of three clones matched those of the BSB and the other three clones had slightly different peak cell densities or growth profiles at the later stage of culture. Using  $kLa$  as SDM criterion may be appropriate for cell screening activities and applicable for the majority of CHO cell lines. Further fine-tuning and the application of other SDM criteria (e.g.,  $pCO_2$ ) may allow the ambr250 to become a better SDM mimic of large-scale bioreactors.

## 2.3. Temperature Considerations in ambr Systems

Each of the ambr15 culture station blocks of 12-vessels can be maintained at different temperatures and most studies report robust temperature control. However, temperature control in the ambr15 can be influenced by the environmental temperature<sup>[12]</sup> and care should be taken to maintain ambient conditions ( $21\text{--}25^\circ\text{C}$ ) as per the manufacturer's recommendations. In comparison, each ambr250 vessel can be maintained at different temperatures and temperature is controlled in a similar manner to larger scale vessels with a liquid filled temperature control jacket.

## 2.4. pH Control Considerations in ambr Systems

One of the disadvantages of the first generation of pH optical sensors utilized in MBRs was the loss of sensitivity to pH over time, leading to the inability of these vessels to control pH later in culture.<sup>[23,39]</sup> However, the pH optical sensors utilized in ambr15 systems have largely been shown to robustly measure pH throughout a production run.

pH control in the ambr15 is achieved by bolus addition of base and by  $CO_2$  sparging. The amount of base to add is determined empirically by equating the volume of base required to change the pH of the cell culture media by 1 U.<sup>[20]</sup> Adjustments to this "base scale factor" may be needed during the production process due to altered media gas state, media composition, and altered buffering capacity. Another consideration is the minimum volume threshold of the ambr15 liquid handler ( $10\text{ }\mu\text{L}$ ), as for some processes a weaker base than the larger scale process may need to be utilized to control pH.<sup>[12]</sup> Overshoots in pH control have also been reported in ambr15 systems that correlate to the addition of a basic feed.<sup>[20]</sup> Such pH drifts can be controlled through tuning  $CO_2$  gas flow limits and the proportional gain in the PI loop.

The ambr250 utilizes a gel electrode single-use probe which are similar to the probe electrodes typically used in larger-scale systems. Moreover, pH control in the ambr250 utilizes PID regulator settings in a similar way to larger-scale vessels.



## 2.5. Feeding and Sampling Considerations

Both the ambr15 and ambr250 system can be integrated with automated cell culture analyzers including the NovaBioProfile FLEX2, Beckman Coulter ViCELL XR, Cedex HiRes, and ambr pH analysis module to allow collection of cell culture data including total and viable cell density, viability, cell diameter, pH, dCO<sub>2</sub>, dO<sub>2</sub>, glucose, lactate, glutamine, glutamate, ammonium, Na<sup>+</sup>, K<sup>+</sup>, Ca<sup>+</sup>, and osmolality. However, careful attention needs to be paid to the sample volume required for such systems (typically 600 µL), especially when conducting daily sampling in small volume (10–15 mL) ambr15 systems.

Indeed, typically volume increases through fed-batch culture duration in larger scale system whereas the volume in ambr15 systems can decrease due to sampling requirements. This can have a detrimental effect on cell culture performance as lower volumes leads to increased gas sparging, which causes aberrant foaming and aggravates dO<sub>2</sub> set-point control. Furthermore, it can take up to 3 hours to analyze all 48 vessels of an ambr15 system which results in a tradeoff between data quantity and experimental throughput.

An article by Rowland-Jones et al.,<sup>[40]</sup> compared different spectroscopic technologies (NIR, Raman and 2D-Fluorescence) to try and resolve this bottleneck. Constraints were imposed so that all analytes were measured in less than 1 h and the sample volume was less than 50 µL. Whereas 2D-Fluorescence was the most suitable technology to quantify ammonium concentration, Raman spectroscopy was the most robust technology for lactate and glucose concentration and was therefore implemented as the at-line platform technology.

Furthermore, once a day sampling may not be appropriate to mimic processes which utilize advanced process analytical technologies that rely on online measurements of cell growth and metabolism.<sup>[41]</sup> Nevertheless, Goldrick et al.,<sup>[42]</sup> recently published an example of advanced process control in ambr15, 7 and 50 L bioreactors by controlling glucose concentration through online measurement of oxygen transfer rate.

The higher *vvm* gas flow rates required in ambr systems can lead to aberrant foam generation which requires additional bolus antifoam additions for adequate control. Increased antifoam additions may impact cell culture performance parameters<sup>[43]</sup> and can create difficulties in downstream processing.<sup>[44]</sup>

Furthermore, only the front set of vessels can be assessed visually for their foam level. Therefore, users typically feed all vessels with antifoam if foam is apparent in the front set of vessels. An interesting observation by Velugula-Yellela et al.<sup>[43]</sup> was that as foaming increased, so did the frequency of variability in the dO<sub>2</sub> measurements. Therefore, variability in dO<sub>2</sub> profile may serve as a potential tool in developing foam control strategy and we recommend antifoam addition when dO<sub>2</sub> variability is high. Both the ambr15 and ambr250 can be utilized to add stepwise feed additions, although only the ambr250 has integrated pumps allowing continuous feed additions. To date, fed-batch processes remain the predominantly reported procedure ran in ambr system and the authors are not aware of any published state-of-the-art intensified high cell density processes of >50 × 10<sup>6</sup> viable cells mL<sup>-1</sup>. At this time no commercially

available cell separation devices can be integrated with the ambr15 and the utilization of open pipe spargers in both ambr systems may provide the required *kLa* for typical fed-batch processes, but this may become limiting for intensified high cell density processes. Nevertheless, Kelly et al.<sup>[45]</sup> have recently published an example of a semi-continuous ambr15 model, which matched metabolic shifts and specific metabolic and protein production rates for a 5 L perfusion process. However, the maximum viable cell density of this process was relatively low (≈20 × 10<sup>6</sup> viable cells mL<sup>-1</sup>). The recent announcement of an ambr250 HT perfusion vessel holds great promise for the future. These vessels are fitted with a multi-hole sparger and integrated cell separation device making them more amenable to intensified high cell density processes.

## 3. Removing the Bottleneck in Experimental Design and Data Analysis with the Application of Advanced Statistical Tools

Even with the application of HT technologies such as the ambr, not all process parameters can be investigated and it is important to generate as much process knowledge as possible with as few experiments as necessary. The first step towards mapping the process design space is to identify process parameters and to assess the risk of each to process robustness. Typically, a formal risk assessment exercise such as Failure Modes and Effect Analysis (FMEA) is conducted to identify factors that could influence CQAs and reduce the number of possible process parameters.<sup>[2,5]</sup> Previous process knowledge can play a key role in this process.<sup>[2,5,19]</sup> Indeed, Janakiraman et al.<sup>[19]</sup> acknowledged the importance of prior process knowledge during the development of their ambr15 SDM of a 15 000 L fed-batch process.

The overwhelming amount of data generated in HT systems calls for efficient experimental design, data handling, and data analysis platforms. Traditional strategies for experimental design rely on the variation of one factor at a time which is laborious, time-consuming, and does not account for synergistic interactions between components. Therefore, Design of Experiments (DoE) is an indispensable tool that facilitates the analysis of a large number of variables simultaneously and helps identify their interactions.<sup>[2,5,46–49]</sup>

The volume of data generated in HT systems can be overwhelming and efficient *in silico* data handling platforms are needed to streamline data entry, processing, and access requirements. Multivariate data analysis (MVDA) techniques can then be helpful to summarize data in a meaningful fashion.

Researchers at Biogen utilized MVDA as a qualification tool to develop an ambr15 scale down model that showed comparability with both manufacturing scale and bench scale.<sup>[19]</sup> Similar MVDA methodology had also been used previously by Biogen to improve a bench scale bioreactor model to match the manufacturing scale process.<sup>[6,7]</sup> Despite the qualification of the scale down model using MVDA the authors noted the importance of applying prior process knowledge (in the form of wet lab characterization experiments), to ensure critical process parameters are closely aligned across different scales.<sup>[19]</sup>

Goldrick et al.<sup>[50]</sup> demonstrated that a combination of DoE studies and MVDA (MLR and PLS-R) can be used to identify and predict product quality heterogeneity (trisulphide bond formation) in ambr15 and 7 L BSBs.

A word of caution is advised when making SDM claims from multivariate summary parameters, such as principal components. While it may be true that principal components easily enable comparisons between datasets, some variables must also be explored individually with classical univariate data analysis (UVDA). In other words; data summarized in a new, reduced variable space of two, or more principal components may appear to match well, but this does not mean that CPPs or CQAs are also matched. It is clear that UVDA alone does not reveal any interactions and therefore both techniques should be utilized.<sup>[51,52]</sup>

The assessment of any SDM's equivalence requires statistical interrogation techniques which aim to compare individual variables and their equivalence or lack thereof. An example is the two one-sided test (TOST), which can be utilized to make a statement about equivalence or non-equivalence of variables. Genzyme have presented a time-series TOST approach for SDM qualification of a micro-carrier perfusion cell culture.<sup>[53]</sup>

FUJIFILM Diosynth Biotechnologies have reported the importance of both UVDA (statistical process control (SPC), *t*-test, *F*-test, Mann-Whitney Test etc.) and MVDA techniques (PCA, PLS, variable importance in projection (VIP), Hotelling's confidence ellipsoids etc.), their roles in validation, and provide the reader with a list of items to consider when qualifying a process in different scales.<sup>[51]</sup>

An example of the utilization of multivariate tools to gain further process understanding during cell culture process development has been provided by Sokolov et al.,<sup>[54]</sup> who utilized a sequential procedure to derive the required information to define further experimentation and decide on the appropriate experimental scale.

In summary, the incorporation of statistical techniques and multivariate modeling tools are indispensable tools that support the analysis of HT experiments and establishment of SDM in major pharmaceutical companies today, of which some few are mentioned in **Figure 2**.

#### **4. Reaching Qualified SDM Status with ambr Systems and Their Role in Accelerating Process Characterization/Validation Studies**

To reach qualified SDM status, ambr process CQAs should match those at the commercial scale and any differences should be well understood. Qualification process robustness can be reinforced by the utilization of media and feed stocks that have been used in commercial scale manufacturing batches. A well-characterized system is crucial for obtaining regulatory approval as can be seen in the example of the first QbD submission by Genentech's Perjeta where this was initially not the case [Kim S., QbD in Biologics: Genentech's success and failure in design space approval, *QbD Works*, 2013, accessed Dec 2017, <<http://qbdworks.com/qbd-biologics-genentechs-success-failure-design-space-approval/>>].

Regulatory authorities welcome submissions which are supported by DoEs coming from small scales such as the

#### **Text box: Regulatory perspectives**

##### **Excerpt from the EC's GMP Annex 15:**

Process validation should establish whether all quality attributes and process parameters, which are considered important for ensuring the validated state and acceptable product quality, can be consistently met by the process. The basis by which process parameters and quality attributes were identified as being critical or non-critical should be clearly documented, taking into account the results of any risk assessment activities. For all products irrespective of the approach used, process knowledge from development studies or other sources should be accessible to the manufacturing site, unless otherwise justified, and be the basis for validation activities.

A much more formal approach for qualification and validation of a process to manufacture medicinal products for human and veterinary use can be found in the FDA's Guidelines for Industry Process Validation: General Principles and Practices<sup>1</sup> and in the EC's (European Commission) Guidelines to Good Manufacturing Practice (EU GMP, EudraLex, Volume 4 and Annexes 2). The topic of process validation may not be discussed in the required depth in this format, some aspects relevant for the purposes of this contribution, namely how SDM studies can be used as part of a process validation strategy, are highlighted here. The EC's GMP Annex 15 (Sections 5.7, 5.10) can be consulted for more information on small scale studies, which support and form the basis of validation activities for licensed products<sup>2</sup>. Following these guidelines with quality risk based principles, the number of full scale process validation batches may be potentially decreased. It is well recognized that for clinical phase material, the requirements for formal process validation are low with the exception of viral clearance for some processes. This offers opportunities to improve a medicinal product while still under investigation, e.g. for clinical trials. It also implies that well-designed SDM studies can be used to set in-process controls for manufacture already in small scale, see EU GMP Annex 13 (Section 16. and 17).<sup>2</sup>

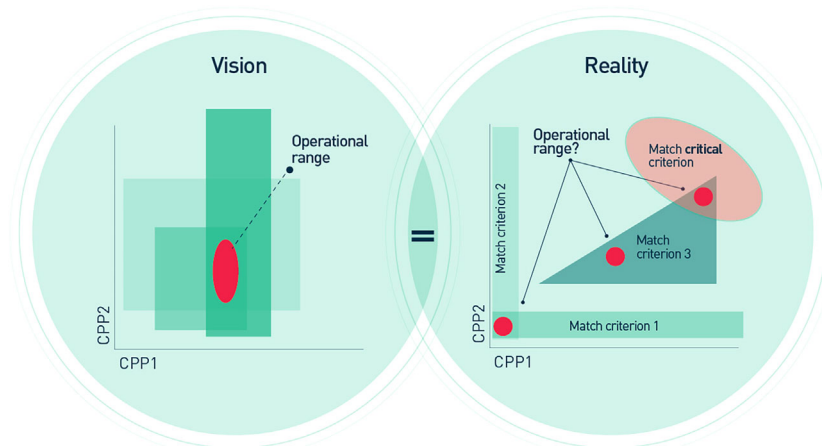
1. FDA, Guidance for industry. Process Validation: General Principles and Practices, FDA, 2011, accessed Dec 2017, <<https://www.fda.gov/downloads/Drugs/Guidances/UCM070336.pdf>>.
2. European Commission, EudraLex – Volume 4–Good Manufacturing Practice (GMP\_ guidelines, European Commission, 2010, accessed Dec 2017, <[https://ec.europa.eu/health/documents/eudralex/vol-4\\_en](https://ec.europa.eu/health/documents/eudralex/vol-4_en)>.



**Figure 2.** Frequently used data analysis tools in industry. PCA (Principal Component Analysis), PLS-R (Partial Least Squares Regression),  $T^2$  (Hotelling's Square), RSP (Response Surface Plots), SPC (Statistical Process Control), Equivalence Testing (TOST, tolerance intervals).

discussed ambr systems. When they are set up to represent a large scale process as closely as possible, these physical models may identify CQAs and the criticalities of process parameters. This may improve process knowledge and help to devise a control strategy to manage residual risk presented by individual or a combination of process parameters (Text box). An important consideration when utilizing ambr systems as SDMs is that ultimately different SDM criteria may be operated for

different scales but these variations must not significantly impact CQAs. Furthermore, the criticality of certain process parameters may vary on a cell line and process specific basis and conflicts may occur between CPPs. For example, a balance between agitation and sparging must be found to supply sufficient oxygenation without cellular stress. Ultimately, the smart selection of SDM criteria should be based on a prioritization of process parameters which most impact CQAs for a given cell line and process. (**Figure 3**).



**Figure 3.** Selection of critical process parameters in ambr systems. A “sweet spot” of process parameters, in which all scale-down mode criteria are matched, may be unattainable. In practice, scale down model criteria are in conflict with each other, however, some exert greater control of critical quality attributes (CQAs) than others and these drive the selection of critical process parameters (CPPs).

Furthermore, what if a large number of process parameters are deemed equally critical and the design criteria cannot be met in a manageable number of experiments? Even if a SDM does not demonstrate equivalence in every single CPP or CQA, it is still possible to address the importance of certain parameters with partial (or sometime worst-case) SDMs [McKnight N., Scale-down model qualification and use in process characterization. *CMC Strategy Forum*, 2013, accessed Dec 2017, <[cymcdn.com/sites/www.casss.org/resource/resmgr/CMC\\_No\\_Am\\_Jan\\_Spkr\\_Slds/2013\\_CMCJ\\_McKnightNathan.pdf](http://cymcdn.com/sites/www.casss.org/resource/resmgr/CMC_No_Am_Jan_Spkr_Slds/2013_CMCJ_McKnightNathan.pdf)>]. Such SDMs can be used to investigate particular subsections of experimental ranges and can be utilized for provocation studies, e.g., effect of  $pCO_2$  profiles, shear stress, or physico-chemical gradients on cell metabolism, viability, and productivity.<sup>[53]</sup> Ultimately, rather than having a “one size fits all SDM” for process characterization and validation studies, ambr systems may play a role as partial SDMs

**Table 1.** Checklist to prepare for regulatory SDM justification.

Question	Answer
How should scale-dependent and scale-independent parameters be selected in the SDM?	Scale-independent parameters such as pH, pO <sub>2</sub> , temperature, nutrient feed addition schedule, seed density, and others should be operated at the set-point conditions of large scale to qualify the SDM. <sup>[18]</sup>  Scale-dependent engineering parameters such as agitation, aeration, vessel pressure, working volume, residence times, geometric similarity, nutrient feed volume, gas stripping, and cell physiology should be comparable in the SDM to ensure comparable performance with large scale. <sup>[13]</sup>
How can equivalence of the SDM be demonstrated?	Equivalence is expected to be demonstrated by overlaying time profiles of variables such as viable cell density, culture viability, bioreactor titer, and others. Metabolic markers such as pCO <sub>2</sub> or specific consumption rate profiles may be included. Those QAs selected for equivalence estimation should be impacted in similar ways both in large scale and in the SDM, otherwise the SDM may not be representative enough for the overall process. <sup>[67]</sup>
Which QAs should be compared in the SDM?	Critical QAs may be known beforehand for a specific product, in which case they have to be included in the SDM comparison. In case they are not known, identification of product related QAs can be driven by those which are known to be influenced by this stage and also by knowledge of the impact in DSP. Frequently, QAs for comparison of small scale and commercial scale are: titre, purity, aggregates, fragments, charge variants, and glycan profile. <sup>[55,68]</sup>
How could comparability of QAs be demonstrated in the SDM?	Compare QA time profiles or end points. Acceptance criteria for comparability in the SDM should not be as strict as if the drug was submitted as a commercial end product. If a small proportion of a large number of QA's identified for the purpose of comparison are demonstrated to have not scaled particularly well, this would not necessarily invalidate the SDM. Instead, it could be shown that some QAs are scale-dependent and which process parameter caused the offset. A justification is appropriate, for instance when conditions were selected that would compromise less important QAs but keep most CQAs within an acceptable range. <sup>[53]</sup>
When can I use a Design Space from my SDM?	A Design Space validation would not end after a singular validation run, instead the DS will be challenged with data on a regular basis for ongoing confirmation. Any process adjustments made at full commercial scale as part of routine manufacture would have to be validated by the SDM in terms of the outputs measured at both scales. Regulatory authorities would anticipate the DS to be adequately and continuously monitored and verified as part of the DS qualification at commercial scale during normal lifecycle. These verifications serve to further qualify the SDM [FDA, Guidance for industry. Process Validation: General Principles and Practices, FDA, 2011, accessed Dec 2017, <a href="https://www.fda.gov/downloads/Drugs/Guidances/UCM070336.pdf">https://www.fda.gov/downloads/Drugs/Guidances/UCM070336.pdf</a> ].

This checklist intentionally does not cover all aspects of SDM, rather it should be a helpful summary of questions and their answers that were agglomerated from various sources over the course of this study.

that allow more targeted studies of the direction and magnitude of particular effects. Regardless of whether ambr systems are utilized as fully miniaturized SDMs or as partial SDMs, both require qualification.

Data from a SDM alone would not be accepted instead of commercial scale process validation, as its role is presumed to support and not to replace process validation activities. This qualified status can only be attained once clinical phase manufacturing data becomes available. However, if the quality of the data is high and a high level of process knowledge can be demonstrated, the SDM may be expected to reduce the burden of full scale validation and support certain claims instead. These claims could be, e.g., proven acceptable ranges or they may permit reduction of number of batches executed for process qualification purposes (PPQ). A checklist of questions and answers was prepared to guide researchers in justifying how they developed and justified their SDM (Table 1).

Many biopharmaceutical companies employ platform processes that are designed to accelerate the timeline to clinical manufacturing.<sup>[56]</sup> These encompass a combination of well-tested components, such as cell line, media/feed strategies, equipment configuration, and scale-up strategy. Ideally, the majority of processes would fit to the qualified platform process and both ambr systems already fit very well into the platform

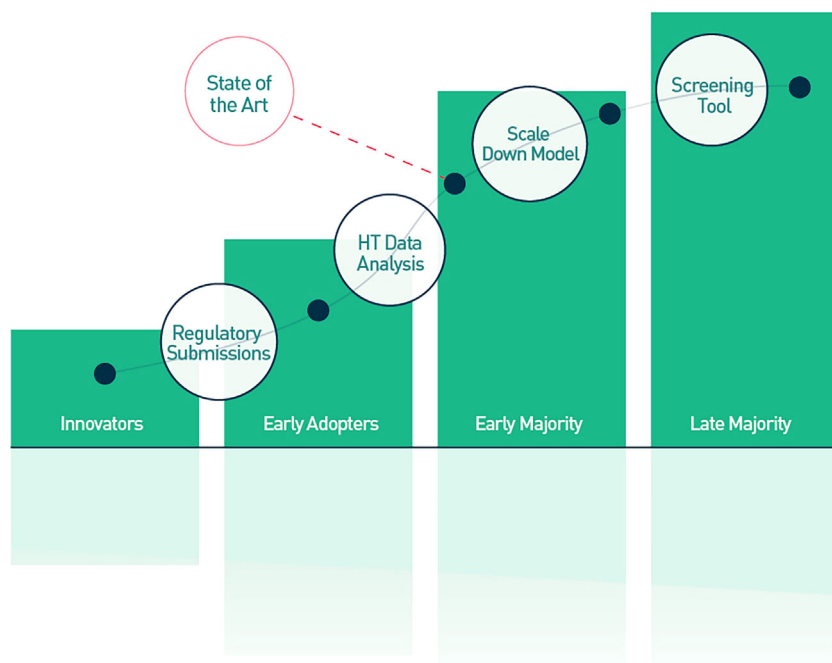
concept as a widely utilized screening tool. Therefore qualified ambr could be utilized to start process characterization studies much earlier (during cell line selection and process optimization) and speed up the development life-cycle of today's drug manufacturing process.

## 5. Increasing Understanding of the Culture Environment at Small and Large Scale

An important pre-requisite for a SDM is that the cell culture environment is matched as closely as possible between small and large scale. This understanding requires a detailed evaluation of both mass transfer (O<sub>2</sub> supply, dCO<sub>2</sub> stripping and bulk mixing) and cellular stress at each scale.

Detailed process knowledge and extensive in vitro characterization of process equipment through the establishment of predictive models and simulation tools based on fundamental engineering principals are necessary to determine matched cell culture performance between scales.<sup>[57]</sup> Computational fluid dynamics (CFD) can also be utilized to provide an in silico visualization of flow patterns, local gradients, and shear sensitive zones.<sup>[32,36,58,59]</sup> Once visualized, mass transfer, and stress heterogeneity between scales can be matched.<sup>[60]</sup>





**Figure 4.** Expected evolution of ambr utilization over time. The biopharmaceutical industry already widely utilizes the ambr as a screening platform. Challenges today are development and validation of scale down models for cGMP manufacturing processes. HT data will require automation and adequate database tools that allow uni- and multivariate statistical analysis. Finally, the innovators of our time will have to demonstrate that the cumulative quality of all work elements leads to a positive regulatory submission, which will firmly establish the ambr as an integral element of biopharmaceutical drug development.

Of interest in the future will be how best to leverage the information provided by –OMICS technologies to help understand the differences in the cellular environment between scales. An example of such an approach has been published by BMS in which a combined metabolomics and proteomic approach identified hypoxia as the cause for differences in process performance differences between a 20 L BSB and a 5000 L bioreactor.<sup>[61]</sup> Another recent example by Alsayyari et al.,<sup>[62]</sup> observed only small differences in overall gene expression between ambr15 and 10 L BSB systems.

Furthermore, whereas classical scale-down criteria include the matching of process set-points such as  $dO_2$ , a more elegant approach may involve setting a lower  $dO_2$  set-point at the small-scale (homogenous mixing environment) to match the average CFD derived/cellular stress at the heterogeneous large-scale (heterogeneous mixing environment).<sup>[63]</sup> This may be a very process specific recommendation, as some cell lines are more sensitive to the same stresses than others. Nevertheless, we believe that properly selecting and reproducing large scale stresses in small scale, either by appropriate physical or computational tools,<sup>[64–66]</sup> will improve our understanding and result in more robust commercial processes.

## 6. Conclusions

MBRs such as the ambr systems are powerful tools for the biopharmaceutical manufacturing industry. However, regardless

of their success and rapid adaptation, they are still often viewed as complementary screening platforms rather than SDMs of commercial scale manufacturing processes. The question therefore remains, how can these systems transition into SDMs?

One challenge that researchers face is that SDM criteria may not be 1:1 comparable between small- and large-scale. Therefore, these novel MBRs require extensive characterization and *also* novel SDM criteria to match CQAs across scales. However, even traditional methods of SDM qualification utilizing BSBs can be challenging and the HT capability of ambr systems enables rapid exploration of the multivariate design space. Therefore, key to the adoption of ambr systems will be the application of advanced risk analysis and statistical tools such as FMEA, DoE, UVDA, and MVDA to remove bottlenecks in experimental design and data analysis.

It is important to note that not all processes may be applicable to utilizing ambr systems as “one-stop shop” SDMs and it is important to fully understand equipment limitations and the relevant CPPs and CQAs that are to be matched. ambr systems may therefore play a role as partial SDMs that allow more targeted studies of the direction and magnitude of particular effects in BSBs. Nevertheless, evidence is already available that suggests that ambr systems can be utilized to represent

certain aspects of manufacturing scale processes. Further process understanding and data generated for HT SDMs should further expand their utility and reduce the resource requirements and time-line for process characterization and validation studies. With this outlook we can look forward to the first regulatory submissions utilizing ambr SDMs that are expected to showcase the best practice in some, if not all disciplines (Figure 4).

## Abbreviations

BSB, bench-scale bioreactor; CFD, computational fluid dynamics; cGMP, current good manufacturing practice; CHO, Chinese hamster ovary; CPP, critical process parameter; CQA, critical quality attribute;  $dCO_2$ , dissolved  $CO_2$ ;  $DO_2$ , dissolved oxygen; DoE, design of experiment; FMEA, failure modes and effects analysis; HT, high-throughput;  $kLa$ , volumetric mass transfer coefficient; mAb, monoclonal antibody; MBR, miniature bioreactor; MLR, multiple linear regression; MVDA, multivariate data analysis; OTR, oxygen transfer rate; PCA, principal component analysis; PLS-R, partial least squares regression;  $P/V$ , power per unit volume; SDM, scale down model; TOST, two one-sided test;  $ts$ , tip speed; UVDA, univariate data analysis;  $vvm$ , vessel volumes per minute.

## Supporting Information

Supporting information is available from the Wiley Online Library or from the author.

## Acknowledgements

The project has received funding from the European Union's Horizon 2020 research and innovation program under the Marie Skłodowska Curie grant agreement No. 643056. We also wish to thank the R&D team at FUJIFILM Diosynth Biotechnologies UK for their interest and warm support for this project. VS and GM conceived the study, VS drafted and wrote the manuscript. JG and GM added subject matter expertise, especially in the data analysis and regulatory sections. LP summarized and added to the manuscript, especially in the SDM sections. JG and GM added useful critique to the manuscript and supervised research activities. The authors gratefully acknowledge the financial support from the EU-Horizon 2020 Marie Skłodowska-Curie Actions (MSCA) ITN project BIORAPID (no. 643056).

## Conflicts of Interest

The authors declare no commercial or financial conflict of interest.

## Keywords

high-throughput systems, miniature bioreactors, process characterization, regulatory qualification, scale-down models

Received: October 17, 2017

Revised: October 16, 2018

Published online: November 26, 2018

- [1] A. S. Rathore, H. Winkle, *J. Nat. Biotechnol.* **2009**, 27, 26.
- [2] S. F. Abu-Absi, L. Yang, P. Thompson, C. Jiang, S. Kandula, B. Schilling, A. A. Shukla, *Biotechnol. Bioeng.* **2010**, 106, 894.
- [3] A. Banerjee, *BioPharm Int.* **2010**, 23, 26.
- [4] M. Harmsen, J. Stofferis, L. Malphettes, *BMC Proc.* **2011**, 5, P70.
- [5] F. Li, Y. Hashimura, R. Pendleton, J. Harms, E. Collins, B. Lee, *Biotechnol. Prog.* **2006**, 22, 696.
- [6] L. Tescione, J. Lambropoulos, M. R. Paranandi, H. Makagiansar, T. Ryll, *Biotechnol. Bioeng.* **2015**, 112, 84.
- [7] V. L. Tsang, A. X. Wang, H. Yusuf-Makagiansar, T. Ryll, *Biotechnol. Prog.* **2014**, 30, 152.
- [8] J. D. Yang, C. Lu, B. Stasny, J. Henley, W. Guinto, C. Gonzalez, J. Gleason, M. Fung, B. Collopy, M. Benjamino, J. Gangi, M. Hanson, E. Ille, *Biotechnol. Bioeng.* **2007**, 98, 141.
- [9] C. S. Alves, A. Gilbert, S. Dalvi, B. St Germain, W. Xie, S. Estes, R. Kshirsagar, T. Ryll, *Biotechnol. Prog.* **2015**, 31, 1201.
- [10] R. Bareither, N. Bargh, R. Oakeshott, K. Watts, D. Pollard, *Biotechnol. Bioeng.* **2013**, 110, 3126.
- [11] F. Delouvroy, G. Le Reverend, B. Fessler, G. Mathy, M. Harmsen, N. Kochanowski, L. Malphettes, *BMC Proc.* **2013**, 7, P73.
- [12] W. T. Hsu, R. P. S. Aulakh, D. L. Traul, I. H. Yuk, *Cytotechnology* **2012**, 64, 667.
- [13] Y. Huang, C. Kwiatkowski, *Pharm. Bioprocess.* **2015**, 3, 397.
- [14] B. J. Kim, J. Diao, M. L. Shuler, *Biotechnol. Prog.* **2012**, 28, 595.
- [15] G. Lewis, R. Lugg, K. Lee, R. Wales, *Bioprocess J.* **2010**, 9, 22.
- [16] S. Moses, A. Manahan, A. Ambrogelly, W. L. W. Ling, *Adv. Biosci. Biotechnol.* **2012**, 3, 918.
- [17] R. Bareither, M. Goldfeld, C. Kistler, A. Tait, N. Bargh, R. Oakeshott, K. O'Neill, L. Hoshan, D. Pollard, *Pharm. Bioprocess.* **2015**, 3, 185.
- [18] F. Delouvroy, G. Siriez, A. V. Tran, L. Mukankurayija, N. Kochanowski, L. Malphettes, *BMC Proc.* **2015**, 9, 78.
- [19] C. Janakiraman, R. Kwiatkowski, R. Kshirsagar, T. Ryll, Y. M. Huang, *Biotechnol. Prog.* **2015**, 31, 1623.
- [20] S. Rameez, S. S. Mostafa, C. Miller, A. A. Shukla, *Biotechnol. Prog.* **2014**, 30, 718.
- [21] P. Xu, C. Clark, T. Ryder, C. Sparks, J. Zhou, M. Wang, R. Russel, C. Scott, *Biotechnol. Prog.* **2017**, 33, 478.
- [22] A. Amanullah, J. M. Otero, M. Mikola, A. Hsu, J. Zhang, J. Aunins, H. B. Schreyer, J. A. Hope, A. P. Russo, *Biotechnol. Bioeng.* **2010**, 106, 57.
- [23] A. Chen, R. Chitta, D. Chang, A. Amanullah, *Biotechnol. Bioeng.* **2009**, 102, 48.
- [24] S. R. Warr, *Methods Mol. Biol.* **2014**, 1104, 149.
- [25] S. Ahuja, S. Jain, K. Ram, *Biotechnol. Prog.* **2015**, 31, 1370.
- [26] M. De Jesus, P. Girard, M. Bourgeois, G. Baumgartner, B. Jacko, H. Amstutz, F. M. Wurm, *Biochem. Eng. J.* **2004**, 17, 217.
- [27] M. Gomez, M. Ambhaikar, L. Zhang, C. J. Huang, *Biotechnol. Prog.* **2017**, 33, 490.
- [28] N. Matsunaga, K. Kano, Y. Maki, T. Dobashi, *J. Biosci. Bioeng.* **2009**, 106, 412.
- [29] Z. Xing, B. M. Kenty, Z. J. Li, S. S. Lee, *Biotechnol. Bioeng.* **2009**, 103, 733.
- [30] S. Xu, L. Hoshan, R. Jiang, B. Gupta, E. Brodean, K. O'Neill, T. C. Seamans, J. Bowers, H. Chen, *Biotechnol. Prog.* **2017**, 33, 1146.
- [31] S. J. Meier, T. A. Hatton, D. I. C. Wand, *Biotechnol. Bioeng.* **1999**, 62, 468.
- [32] A. W. Nienow, *Cytotechnology* **2006**, 50, 9.
- [33] D. M. Marks, *Cytotechnology* **2003**, 103, 733.
- [34] V. Restelli, M. D. Wang, N. Huzel, H. Perreault, M. Butler, *Biotechnol. Bioeng.* **2006**, 94, 481.
- [35] E. Trummer, K. Fauland, S. Seidinger, K. Schriebl, C. Lattenmayer, R. Kunert, K. Vorauer-Uhl, R. Weik, N. Borth, H. Katinger, D. Müller, *Biotechnol. Bioeng.* **2006**, 94, 1033.
- [36] A. W. Nienow, C. D. Rielly, K. Brosnan, N. Bargh, K. Lee, K. Coopman, C. Hewitt, *Biochem. Eng. J.* **2013**, 76, 25.
- [37] D. de Wilde, T. Dreher, C. Zahn, U. Husemann, G. Greller, T. Adams, C. Fenge, *BioProcess Int.* **2014**, 12(8)s.
- [38] L. K. Ju, G. G. Chase, *Bioprocess. Eng.* **1992**, 8, 49.
- [39] R. Bareither, D. Pollard, *Biotechnol. Prog.* **2011**, 27, 2.
- [40] R. C. Rowland-Jones, F. van den Berg, A. J. Racher, E. B. Martin, C. Jaques, *Biotechnol. Prog.* **2017**, 33, 337.
- [41] A. Zhang, V. L. Tsang, B. Moore, V. Shen, Y. M. Huang, R. Kshirsagar, T. Ryll, *Biotechnol. Bioeng.* **2015**, 112, 2495.
- [42] S. Goldrick, K. Lee, C. Spencer, W. Holmes, M. Kuiper, R. Turner, S. S. Farid, *Biotechnol. J.* **2017**, 13, e1700607.
- [43] S. R. Velugula-Yellela, A. Williams, N. Trunfio, C. J. Hsu, B. Chavez, S. Yoon, C. Agarabi, *Biotechnol. Prog.* **2018**, 34, 262.
- [44] W. C. McGregor, J. F. Weaver, S. P. Tansey, *Biotechnol. Bioeng.* **1988**, 31, 385.
- [45] W. Kelly, S. Veigne, X. Li, S. S. Subramanian, Z. Huang, E. Schaefer, *Biotechnol. Prog.* **2018**, 34, 420.
- [46] C. Grimm, W. Kusser, B. Lee, G. Bremer, A. Bossie, *BMC Proc.* **2015**, 9, P15.
- [47] M. Tai, A. Ly, I. Leung, G. Nayar, *Biotechnol. Prog.* **2015**, 31, 1388.
- [48] E. B. Moran, S. T. McGowen, J. M. McGuire, J. E. Frankland, I. A. Oyebeade, W. Waller, L. C. Archer, L. O. Morris, J. Pandya, S. R. Nathan, L. Smith, M. L. Cadette, J. T. Michalowski, *Biotechnol. Bioeng.* **2000**, 69, 242.
- [49] M. Shivhare, G. McCreath, *BioProcess Int.* **2010**, 8, 22.
- [50] S. Goldrick, W. Holmes, N. J. Bond, G. Lewis, M. Kuiper, R. Turner, S. S. Farid, *Biotechnol. Bioeng.* **2017**, 114, 2222.
- [51] G. McCreath, M. Shivhare, *EBR* **2011**, Autumn 62.
- [52] M. Sokolov, M. Soos, B. Neunstoecklin, M. Morbidelli, A. Butté, R. Leardi, T. Solacroup, M. Stettler, H. Broly, *Biotechnol. Prog.* **2015**, 31, 1633.
- [53] C. DeCesare, M. Yu, J. Yin, W. Zhou, C. Hwang, J. Tengtrakool, K. Konstantinov, *Bioprocess Int.* **2016**, 14, 18.

- [54] M. Sokolov, M. Morbidelli, A. Butté, J. Sousquet, H. Broly, *Biotechnol. J.* **2018**, *13*, e201700461.
- [55] M. Brunner, J. Fricke, P. Kroll, C. Herwig, *Bioprocess Biosyst. Eng.* **2017**, *40*, 251.
- [56] F. Li, N. Vijayasankaran, A. Y. Shen, R. Kiss, A. Amanullah, *mAbs* **2010**, *2*, 466.
- [57] C. Sieblist, M. Jenzsch, M. Pohlscheidt, *Cytotechnology* **2016**, *68*, 1381.
- [58] D. W. Huttmacher, H. Singh, *Trends Biotechnol.* **2008**, *26*, 166.
- [59] A. O. O. Odeleye, D. T. J. Marsh, M. D. Osborne, G. J. Lye, M. Micheletti, *Chem. Eng. Sci.* **2014**, *111*, 299.
- [60] J. Wutz, A. Lapin, F. Siebler, J. E. Schäfer, *Eng. Life Sci.* **2016**, *16*, 633.
- [61] Y. Gao, S. Ray, S. Dai, A. R. Ivanov, N. R. Abu-Absi, A. M. Lewis, Z. Huang, Z. Xing, M. C. Borys, Z. J. Li, B. L. Karger, *Biotechnol. J.* **2016**, *11*, 1190.
- [62] A. A. Alsayyari, X. Pan, C. Dalm, J. W. van der Veen, N. Vriezen, J. A. Hageman, R. H. Wijffels, D. E. Martens, *J. Biotechnol.* **2018**, *279*, 61.
- [63] A. M. Lewis, W. D. Croughan, N. Aranibar, A. G. Lee, B. Warrack, N. R. Abu-Absi, R. Patel, B. Drew, M. C. Borys, M. D. Reily, Z. J. Li, *PLoS ONE* **2016**, *11*, e0157111.
- [64] R. Spann, A. E. Lantz, K. V. Gernaey, G. Sin, *Ref. Mod. Chem. Mol. Sci. Chem. Eng.* **2018**, *1*. <https://doi.org/10.1016/B978-0-12-409547-2.14356-2>.
- [65] E. Anane, A. Sawatzki, P. Neubauer, M. N. Cruz-Bournazou, *J. Chem. Technol. Biotechnol.* **2018**, 0268. <https://onlinelibrary.wiley.com/doi/abs/10.1002/jctb.5798>.
- [66] C. Haringa, W. Tang, G. Wang, A. T. Deshmukh, W. A. van Winden, J. Chu, W. M. van Gulik, J. J. Heijnen, R. F. Mudde, H. J. Noorman, *J. Chem. Eng. Sci.* **2018**, *175*, 12.
- [67] M. Brunner, P. Braun, P. Doppler, C. Posch, D. Behrens, C. Herwig, J. Fricke, *Biotechnol. J.* **2017**, *12*, <https://doi.org/10.1002/biot.201600633>.
- [68] D. Brühlmann, M. Jordan, J. Hemberger, M. Sauer, M. Stettler, H. Broly, *Biotechnol. Prog.* **2015**, *31*, 615.