Process Intensification Using a One-Step Inoculum Production and High-Seeded Fed-Batch Processes

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Various approaches to process intensification are currently being investigated to ensure time and cost savings when producing biopharmaceuticals. In the present study, a one-step inoculum production was established based on an ultra-high cell density working cell bank with immunoglobulin G producing Chinese hamster ovary cells. Cryovials were used for direct inoculation of 1-L wave-mixed perfusion bioreactors. When reaching around $180 \cdot 10^6$ cells mL^{-1} in N–1 perfusion mode, low-seed and high-seed fed-batch experiments in shake flasks were inoculated, as was the case for 250-mL stirred single-use bioreactors. Additionally, proof-of-concept runs at 50-L and 200-L scale were successfully performed. The intensification approach presented allows manufacturing capacity to be increased by up to 50 %.

Keywords: Chinese hamster ovary cells, Monoclonal antibody, N–1 perfusion, Ultra-high cell density

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1 Introduction

Monoclonal antibodies (mAbs) are often used to treat cancer, as well as autoimmune and infectious diseases. The high costs involved in such treatments are related, among others, to the production costs of the mAbs, which are mainly produced using Chinese hamster ovary (CHO) cells. The prices of mAb treatments range from an average of approx. $ 15 600 annually for the treatment of cardiological and endocrinological diseases to approx. $ 142 800 for the treatment of oncological and hematological diseases [1]. Production costs can be reduced by process intensification with a focus on more efficient process design [2]. In the area of upstream processing of mAb production processes, the improvement of inoculum production by reducing the time or the number of steps of the seed train, and the increase of productivity offer potential for a significant improvement.

The inoculum production time can be shortened by large volume and by high cell density working cell banks (WCBs) [3–11]. For example, Heidemann et al. [4] established a large volume WCB with 50–100 mL volume and a freezing cell density of $20–40 \cdot 10^6$ cells mL^{-1} in cryobags. Tao et al. [7] and Müller et al. [11] worked with freezing densities of 100 and $260 \cdot 10^6$ cells mL^{-1}, respectively. These two procedures allow the small-scale inoculum production steps to be skipped.

High cell density inoculum cultures which are realized by using the perfusion mode offer an opportunity to replace one or several process steps and thus to shorten the seed train. By continuously feeding fresh medium and removing old medium, high cell densities of more than $40 \cdot 10^6$ cells mL^{-1} and even up to $238 \cdot 10^6$ cells mL^{-1} can be achieved [2, 5, 11–16]. Compared to standard inoculum production, cell densities of up to 40 times higher are generated. Consequently, such ultra-high cell density (UHCD) precultures enable the reduction of the size of the inoculum production systems as the production bioreactor can be inoculated with significantly lower inoculum volumes. The combination of intensified cell banks with N–1 perfusion enables one-step inoculum production, which was already proposed by Heidemann et al. in 2002, using the above-mentioned high-volume WCB with a modified inoculum production bioreactor run in perfusion mode [4], and by Cytiva as an application note, using high freezing cell densities and N–1 perfusion in a wave-mixed bag [9].

While the first process intensification approaches described refer to the seed train, improvements can also be made in the mAb production process. Industrial mAb production is usually realized in fed-batch mode [17]. Classically, the production bioreactor is inoculated with low cell densities in the range of 0.2 to $1.0 \cdot 10^6$ cells mL^{-1} [18]. In order to avoid the unproductive growth phase of the cells at the beginning of cultivation, and thus to shorten the production process, inoculation of the process with high-cell densities of $4–10 \cdot 10^6$ cells mL^{-1} is possible [18]. Using this

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intensification approach, Müller et al. [19] were able to achieve a mAb titer of 3.2 g L^{-1} after only 8.5 d instead of 12 d. In this case, the production bioreactor was inoculated with 5.0 instead of $0.3 \cdot 10^6$ cells mL^{-1}. With inoculation of a 40 times higher cell density ($8.0 \cdot 10^6$ cells mL^{-1}) Padawer et al. [20] reduced their process time by one third. A similar reduction of the process time was obtained by Yang et al. [21] who were able to achieve 5 g L^{-1} immunoglobulin G (IgG) after only 12 d by increasing the inoculation cell density to $10 \cdot 10^6$ cells mL^{-1}. The same cell density was used by Mahé et al. [22], who were able to increase the titer by 80 % in a 14-day mAb production process.

The present study aimed at a significant intensification of the entire upstream process, by combination of three intensification methods: (i) the use of a previously established UHCD-WCB [11], (ii) an N–1 perfusion process, and (iii) a high-seed fed-batch approach (Fig. 1). In the N–1 perfusion process, viable cell densities (VCDs) above $150 \cdot 10^6$ cells mL^{-1} were targeted in order to subsequently inoculate the fed-batch experiments. On the one hand, the presented approach can shorten and simplify inoculum production compared to the standard methods, and on the other hand, the production process can be either shortened or, alternatively, higher product titers can be achieved.

2 Material and Methods

2.1 Cell Line and Media

An IgG-producing ExpiCHO-S cell line (Gibco) was used. All perfusion experiments were conducted with High-Intensity Perfusion CHO Medium (Gibco), at a concentration of 0.66× for the start medium and of 1× for the medium added during the perfusion mode. The medium was supplemented with 4 mmol L^{-1} L-glutamine (Sigma-Aldrich) and 0.1 % Anti-Clumping Agent (Gibco). In contrast, for standard inoculum production, Efficient-Pro Medium (Gibco) containing 6 mmol L^{-1} L-glutamine and 0.1 % Anti-Clumping Agent was used. The fed-batch mAb production experiments were executed with Efficient-Pro Medium (Gibco), supplemented with 6 mmol L^{-1} L-glutamine and 0.1 % Anti-Clumping Agent, and Efficient-Pro Feed 2 (Gibco). The feeding procedure is described in detail in Sect. 2.4.

2.2 Standard Inoculum Production

Cells were thawed from a cryovial containing $15 \cdot 10^6$ cells mL^{-1}, then transferred to a 125-mL single-use shake flask (Corning) containing 40 mL medium, and finally expanded for 7 d. Cells were passaged every 2nd or 3rd day with a VCD of 0.3–0.5 $\cdot 10^6$ cells mL^{-1}. For this purpose, 250- and 500-mL single-use shake flasks (Corning) were used with working volumes of 80 and 160 mL, respectively. Shake flasks were incubated in a shaking incubator with a shaking speed of 120 rpm at an amplitude of 25 mm, 37 °C, 8 % CO_2, and 80 % relative humidity.

2.3 Intensified Inoculum Production

For intensified inoculum production, a 4.5-mL cryovial from the UHCD-WCB containing $260 \cdot 10^6$ cells mL^{-1} was thawed and transferred directly into a 2-L wave-mixed single-use bioreactor with internal filter membrane (Cellbag, 2 L, BC10, pHOPT, DOOPT II and Perfusion; Cytiva) containing 1 L medium for cultivations P01 and P02. For P03, a 10-L wave-mixed single-use bioreactor with an internal filter membrane (Flexsafe RM 10 L perfusion pro 1.2 µm, Sartorius) and a working volume of 2 L was inoculated with two 4.5-mL cryovials. Cultivations were carried out with the Ready to Process Wave 25 control unit from GE Healthcare (now Cytiva). Process parameters of the perfusion cultivations are described in [11]. After starting perfusion mode, the aim was that the cell-specific perfusion rate (CSPR) would not decrease below 55 pL $cell^{-1}d^{-1}$ until the perfusion rate D reached a maximum of 7 vvd. Per liter of working volume, 20 L perfusion medium was

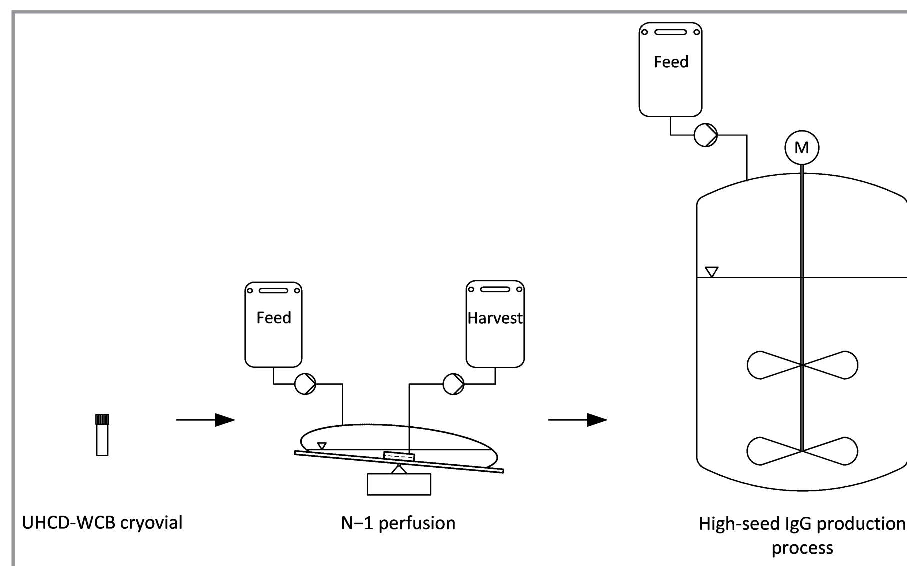

Figure 1. Schematic representation of the intensified approach described in this study. Three intensification methods were combined: a UHCD-WCB, an N–1 perfusion process, and a high-seed IgG production process.

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prepared. Daily samples were taken on days 0 and 1. With the start of the perfusion mode 1.6–1.9 d after inoculation, when >$3 \cdot 10^6$ cells mL^{-1} had been reached, samples were taken twice daily. Cells were harvested after approx. 7.5 d to inoculate fed-batch experiments.

2.4 IgG Production Experiments

As already mentioned, IgG production experiments were performed in fed-batch mode. Four different types of culture systems were used: (i) 250-mL single-use shake flasks (Corning) with a working volume of maximum 54 mL, (ii) ambr 250 modular stirred single-use bioreactor vessels (Sartorius) with a working volume of maximum 250 mL, (iii) the BIOSTAT STR 50 L (Sartorius) with a Flexsafe STR 50 L bag and a working volume of maximum 50 L, and (iv) the BIOSTAT STR 200 L (Sartorius) with a Flexsafe STR 200 L bag and a working volume of maximum 200 L.

Fed-batch experiments with $0.3 \cdot 10^6$ cells mL^{-1} were performed in shake flasks to compare standard and intensified inoculum production. Afterward, in shake flasks as well as ambr vessels, low-seed experiments with 0.3 and $1 \cdot 10^6$ cells mL^{-1}, and high-seed experiments with 5 and $10 \cdot 10^6$ cells mL^{-1}, were performed with intensified inoculum production. At pilot scale, the 50-L bioreactor was inoculated with high-seed ($5.4 \cdot 10^6$ cells mL^{-1}) and the 200-L bioreactor with low-seed cell densities ($1.5 \cdot 10^6$ cells mL^{-1}); both inocula were produced with the intensified method. Samples were taken daily for atline analyses. Feeding was started on day 3 for inoculation with $0.3 \cdot 10^6$ cells mL^{-1}, on day 2 for inoculation with $1 \cdot 10^6$ cells mL^{-1}, and on day 0 for high-seeded experiments. The feed medium was added as a bolus of 2 vol % of the current working volume daily after sampling for a total of 12 d. In addition, a 450 g L^{-1} glucose solution was added to keep the glucose concentration in the bioreactors above 2 g L^{-1}. Shake flasks were incubated in a shaking incubator with 150 rpm shaking speed at an amplitude of 50 mm, 37 °C, 8 % CO_2, and 80 % relative humidity. For the stirred bioreactors, cultivations were performed at 37 °C, with overlay aeration of 0.1 vvm, pH $\leq$ 7.2 controlled by addition of CO_2, and dissolved oxygen controlled to $\geq$ 40 % through the addition of O_2. Stirring speed was adjusted to keep a constant power input of 20 W m^{-3}. Antifoam C (Sigma-Aldrich) was added by a control loop in the ambr system and manually in the pilot-scale bioreactors. Prior to use, Antifoam C was diluted 1:10 in the ambr and 1:1000 for the pilot-scale bioreactors. Due to already known issues with foam control in the ambr system with the used cells and media, the exhaust air outlet of the vessels was equipped with foam traps (50 mL sampling tubes).

2.5 Analytics

The samples of the perfusion and fed-batch experiments were analyzed in a Cedex HiRes analyzer (Roche Diagnostics) for VCD, total cell density, viability, cell diameter, compactness, and aggregation rate. The concentrations of glucose, glutamine, ammonium, lactate, and IgG were determined using a Cedex Bio analyzer (Roche Diagnostics).

3 Results and Discussion

3.1 UHCD-WCB-Based N–1 Perfusion

Three perfusion cultivations were used as inocula production for the fed-batch experiments performed in this study, each inoculated with 4.5-mL UHCD-WCB cryovials. The cultivations with working volumes of 1 L (P01 and P02) and 2 L (P03) grew comparably and reached a VCD_{max} of approx. $180 \cdot 10^6$ cells mL^{-1} with a viability >99 % after 7.5 d (Fig. 2a). As described previously, the cells had a short lag phase after direct inoculation from a UHCD-WCB cryovial [11] and the exponential growth phase started accordingly only from day 1. Between day 1 and the end of cultivation, the specific growth rate was approx. $0.028 \pm 0.006\ h^{-1}$ for all three cultivations.

Due to the comparable growth, the perfusion rate D and the CSPR were also comparable (Fig. 2b). After 6.5 d, the maximum perfusion rate of 7 vvd was set for the last 24 h in all three experiments. Up to this point, the CSPR was maintained above 50 pL $cell^{-1}d^{-1}$. According to the subsequent limitation, the CSPR decreased on the last day but stayed at $\geq$37 pL $cell^{-1}d^{-1}$ until the time of cell harvest.

3.2 Effect of Inoculum Production Method on Fed-Batch Experiments

To test the suitability of the perfusion-based one-step inoculum production for the inoculation of fed-batch processes, experiments were carried out in shake flasks with the standard and the intensified inoculum production method. The growth curves and viability time courses as well as the concentration time course of IgG are shown in Fig. 3.

The shake flasks inoculated with perfusion cells had a slightly prolonged lag phase (Fig. 3a), resulting in a time-shifted growth curve. The growth rate on the first day was 0.025 h^{-1} when using perfusion cells, 33 % less than in the shake flasks using the standard inoculum production (0.037 h^{-1}). Afterwards, the growth rates were comparable. The maximum VCDs achieved were similar, with standard inoculum production reaching $19.0 \pm 2.0 \cdot 10^6$ cells mL^{-1} after 6 d and with intensified inoculum production $19.9 \pm 2.7 \cdot 10^6$ cells mL^{-1} after 9 d. At the time of termination after 15 d, viability was still high at 93.6 ± 3.1 % (standard) and 96.6 ± 0.2 % (intensified), respectively. It is likely that the lag phase can be attributed to the steps required from cell harvest from the perfusion bag up until inoculation of the shake flasks. A 50-mL sampling tube was welded

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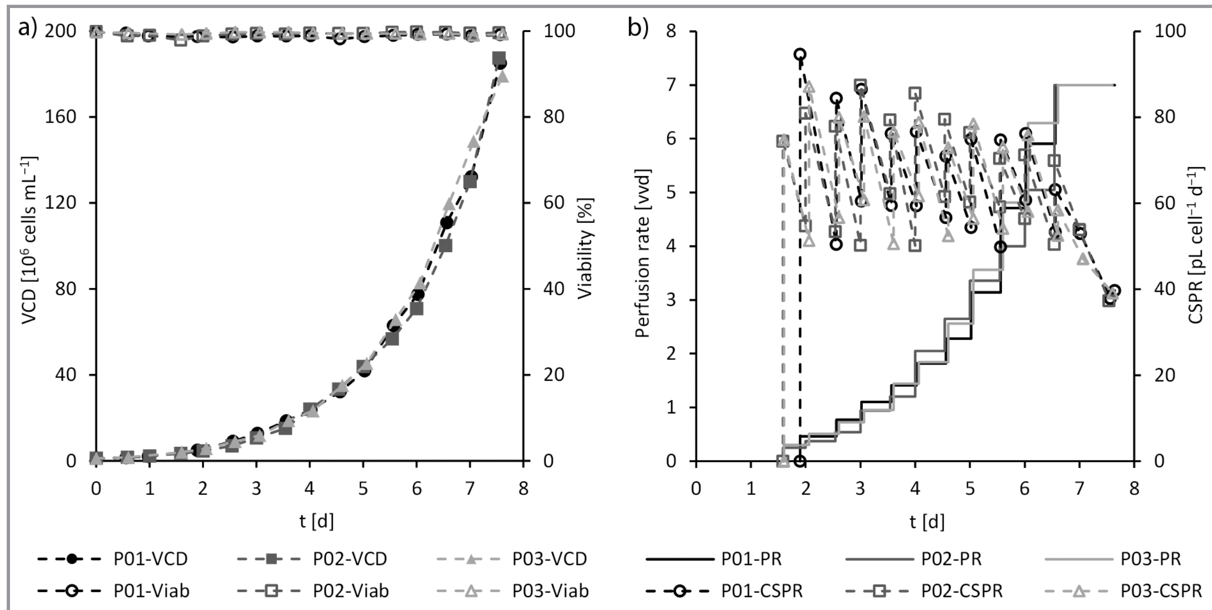

Figure 2. Time course of a) VCD and viability (Viab), and b) perfusion rate (PR) and CSPR during the N–1 perfusion experiments.

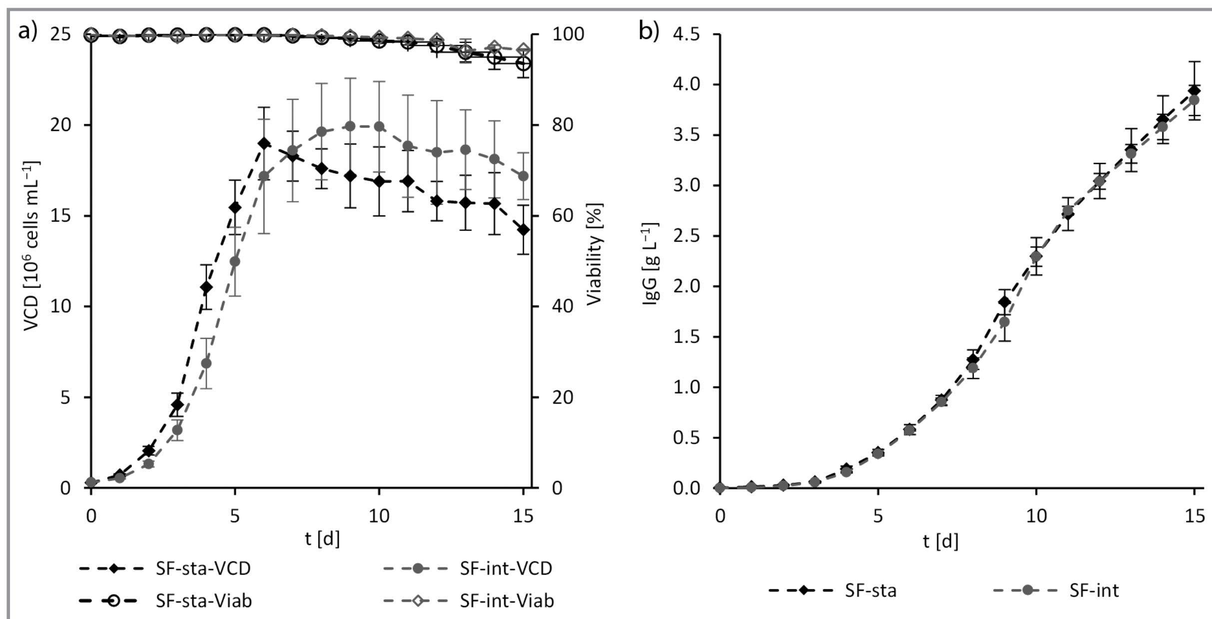

Figure 3. Time course of a) VCD and viability (Viab), and b) IgG concentration during the fed-batch experiments with standard (sta) and intensified (int) inoculum production in shake flasks (SF). $N = 8$ for SF-sta and $N = 4$ for SF-int.

to the wave-mixed bag, cell suspension was transferred and diluted in a shake flask, after which the VCD was measured once again to enable accurate inoculation before the shake flasks were finally inoculated. During these steps, there was at least partial oxygen limitation due to the high VCD, which could have led to this lag phase. Also, the change of medium from the perfusion medium to the Efficient-Pro Medium may have required a short adaptation of the cells.

Despite the delayed growth, no significant differences in IgG production were observed (Fig. 3b). After 15 d, the IgG concentration was approx. 3.9 $g\,L^{-1}$ in both cases. Müller et al. [19] also obtained comparable results in fed-batch experiments inoculated from an N–1 perfusion and a standard inoculum production.

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3.3 Effect of Seeding Density on Fed-Batch Experiments

Since growth and IgG formation showed that the intensified inoculum production leads to comparable results to the standard method, the next step was to investigate the intensification of the production process itself by high inoculation VCDs. Low-seed (0.3 and $1 \cdot 10^6$ cells mL^{-1}) and high-seed (5 and $10 \cdot 10^6$ cells mL^{-1}) cultivations were performed in shake flasks. The feeding procedure is described in detail in Sect. 2.4. The time courses of VCD, viability, and IgG concentration are shown in Fig. 4.

As expected, VCD_{max} was reached earlier, and also higher VCDs were obtained in the cultivations with higher seeding cell densities (Fig. 4a). While the VCD_{max} of approx. $20 \cdot 10^6$ cells mL^{-1} was obtained in the culture with the lowest inoculum after only 9 d, the cultivation time to the VCD_{max} could be reduced to 3 d with the highest inoculum. In this case, even the cell density was 38 % higher compared to the culture with the lowest inoculum. In all cases, the viability was still >90 % at the end of cultivation. Furthermore, with higher seeding VCDs, higher IgG titers could be achieved (Fig. 4b). While 3.84 ± 0.15 g L^{-1} IgG was produced within 15 d in the culture with the lowest inoculum, 4.16 ± 0.09 g L^{-1} and 4.42 ± 0.11 g L^{-1} IgG, i.e., an 8–15 % higher yield was obtained by the cultures with high-seed inocula after only 12 d. To achieve the same amount of IgG as after 15 d in the standard fed-batch process with the lowest inoculum, only 11 d were necessary when starting with $5 \cdot 10^6$ cells mL^{-1} and only 10 d with the highest seeding density, which corresponds to a time saving of up to 33 %. Previous publications characterizing high-seeded fed-batch processes with other cell lines also showed that the inoculation VCD is one of the critical factors for the peak VCD and also reported similar findings regarding time savings or higher product titers [19–22].

The fed-batch processes established in shake flasks were repeated in the ambr 250 modular stirred single-use bioreactor system. Despite operational challenges (overfoaming), comparable VCDs and IgG titers were obtained in the ambr 250 experiments (data not shown).

3.4 Scale-Up to Pilot-Scale

To transfer the approaches to pilot-scale, the stirred single-use bioreactors with maximum working volumes of 50 L and 200 L were inoculated with $5.4 \cdot 10^6$ cells mL^{-1} and $1.5 \cdot 10^6$ cells mL^{-1}, respectively. Fig. 5 shows the VCD, viability, and IgG concentration curves. In the bioreactors, maximum VCDs of $23.3 \cdot 10^6$ cells mL^{-1} after 4 d (50-L STR) and $22.1 \cdot 10^6$ cells mL^{-1} after 7 d (200-L STR) were reached before the stationary phase occurred, during which the VCD slowly decreased to approx. $17.5 \cdot 10^6$ cells mL^{-1} (Fig. 5a). The viability at the time of termination was >90 % in both experiments. The product formation was similar to the experiments in shake flasks and ambr vessels with comparable seeding VCD. After 12 d, a maximum IgG concentration of 4.24 g L^{-1} was reached in the 50-L bioreactor, and 3.60 g L^{-1} after 13 d in the 200-L STR. These results highlight the improvements in the production process achieved in shake flasks and ambr vessels by high-seeding approaches.

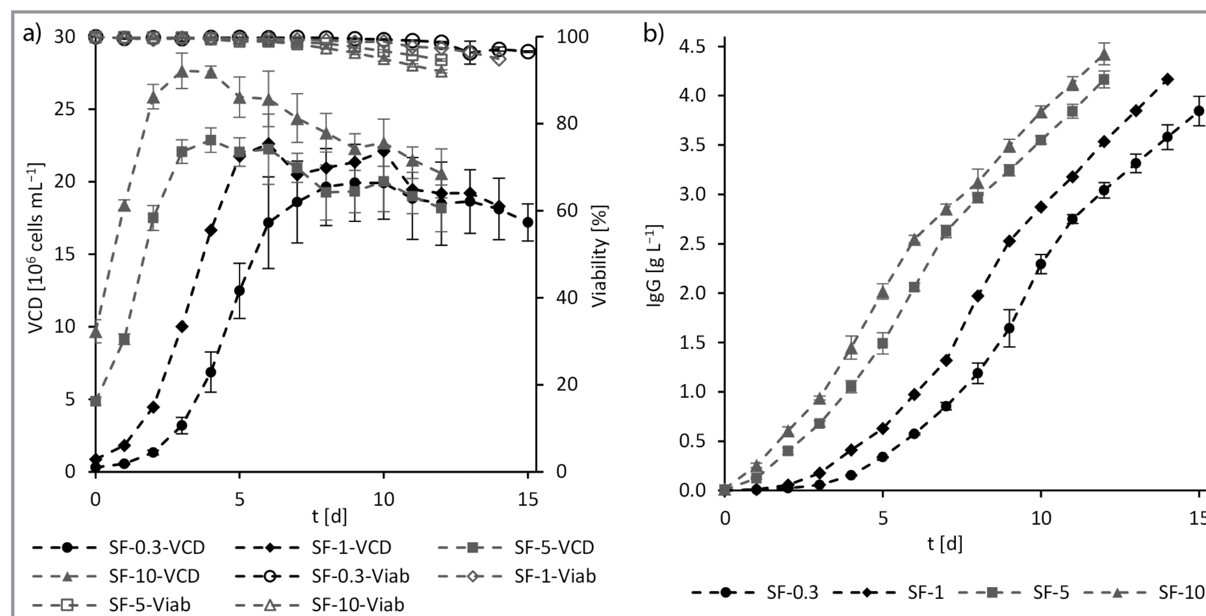

Figure 4. Time course of a) VCD and viability (Viab), and b) IgG concentration during the fed-batch experiments in shake flasks (SF), inoculated with different VCDs (0.3, 1, 5, and $10 \cdot 10^6$ cells mL^{-1}). $N = 4$ for SF-0.3, SF-5, and SF-10; $N = 1$ for SF-1.

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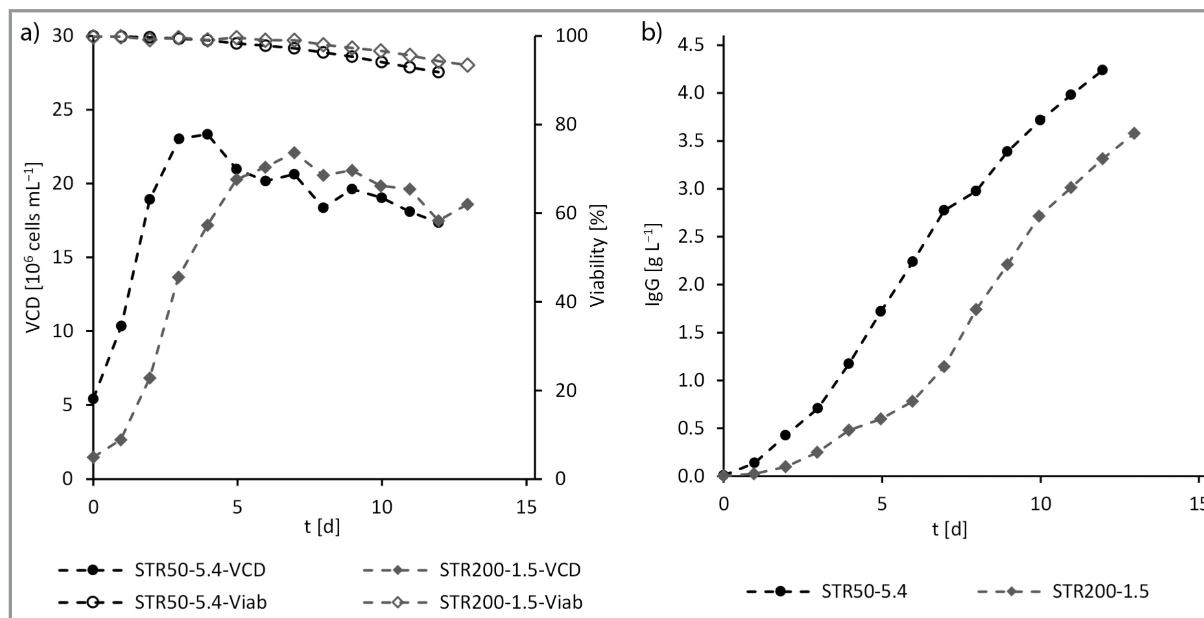

Figure 5. Time course of a) VCD and viability (Viab), and b) IgG concentration during the fed-batch experiments in the 50-L STR, inoculated with $5.4 \cdot 10^6$ cells mL^{-1} (STR50-5.4), and in the 200-L STR, inoculated with $1.5 \cdot 10^6$ cells mL^{-1} (STR200-1.5). $N = 1$ for STR50-5.4 and STR200-1.5.

4 Conclusions

Based on the results achieved in shake flasks, an existing production process inoculated with $0.3 \cdot 10^6$ cells mL^{-1} can be intensified by increasing the seeding VCD. Compared to the standard fed-batch process inoculated with $0.3 \cdot 10^6$ cells mL^{-1}, which was harvested after 15 d, inoculating with $5 \cdot 10^6$ cells mL^{-1} resulted in a time saving of 20 % with a simultaneous IgG titer increase of 8 % (harvest after 12 d). A duration of 12 d instead of 15 d means that 25 % more production runs can be carried out in the same time. With additional consideration of the IgG titer increase, this results in 35 % increase in manufacturing capacity (Tab. 1).

A comparable increase in manufacturing capacity could be achieved by harvesting the high-seeded fed-batch already after 11 d and thus achieving the same IgG titer as in the standard process in 27 % less time. Accordingly, 36 % more runs can be performed in the production bioreactor. Based on a starting VCD of $10 \cdot 10^6$ cells mL^{-1}, the manufacturing capacity can be increased by 44 % in the 12-day process; a shortened process in which the same IgG titer as in the 15-day comparison process can be achieved within 10 d would enable an increase of 50 %.

For the 50-L scale, the use of UHCD-WCB-based one-step inoculum production also means that only 8 d of inoculum production are necessary to produce the cells for a high-seed production process with $5 \cdot 10^6$ cells mL^{-1}, instead of 11 d with a standard seed train with shake flasks and a wave-mixed bag (Tab. 2). If the N–1 perfusion process can be transferred to the largest available wave-mixed bags with an internal filter membrane and with a working volume of up to 25 L (Cytiva/Sartorius), a bioreactor such as this could

Table 1. Influence of seeding VCD on production time, IgG titer, and manufacturing capacity.

Seeding VCD [10^6 cells mL^{-1}]	Day of harvest	Final IgG titer [g L^{-1}]	Time saving$^{a)}$ [%]	Increase IgG titer$^{a)}$ [%]	Increase production runs per time$^{a)}$ [%]	Increase manufacturing capacity$^{a),b)}$ [%]
0.3 ($N = 4$)	15	3.84 ± 0.15	–	–	–	–
5 ($N = 4$)	12	4.16 ± 0.09	20	8	25	35
	11	3.84 ± 0.07	27	0	36	36
10 ($N = 4$)	12	4.42 ± 0.11	20	15	25	44
	10	3.84 ± 0.07	33	0	50	50

a) compared to the standard fed-batch process inoculated with $0.3 \cdot 10^6$ cells mL^{-1} b) combination of increase in IgG titer and increase in production runs.

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Table 2. Comparison of different seed train scenarios for a production process with 50 L and 1 m^3.

Cryovial	Inoc. Production bioreactors	Growth rate during inoc. production[a)] [h^{-1}]	V_{start} production bioreactor [L]	V_{max} production bioreactor [L]	VCD_{start} production bioreactor [cells mL^{-1}]	Inoc. production time[b)] [d]
1.5 mL, $10 \cdot 10^6$ cells mL^{-1}	Shake flasks, wave-mixed	0.035	35	50	$0.3 \cdot 10^6$	7.8
					$5 \cdot 10^6$	11.1
4.5 mL, $260 \cdot 10^6$ cells mL^{-1}	Wave-mixed (N–1 perfusion)	0.027	35	50	$0.3 \cdot 10^6$	3.4
					$5 \cdot 10^6$	7.7
1.5 mL, $10 \cdot 10^6$ cells mL^{-1}	Shake flasks, wave-mixed, stirred	0.035	700	1000	$0.3 \cdot 10^6$	11.4
					$5 \cdot 10^6$	14.7
4.5 mL, $260 \cdot 10^6$ cells mL^{-1}	Wave-mixed (N–1 perfusion)	0.027	700	1000	$0.3 \cdot 10^6$	8.0
					$5 \cdot 10^6$	12.4

a) empirical data for standard inoculum production and N–1 perfusion; b) assuming optimal yield (no loss of cells during inoculum production). Inoc. = Inoculum.

be inoculated with only one cryovial of the UHCD-WCB at the minimum working volume (5 L), gradually filled up to 25 L, and then used for high-seed inoculation of a 1-m^3 bioreactor. Assuming a growth rate of 0.027 h^{-1} on average, the one-step inoculum production would take approximately 12.5 d. To produce the same amount of cells in a conventional inoculum production, assuming a cryovial with 1.5 mL and $10 \cdot 10^6$ cells mL^{-1} and a specific growth rate of 0.035 h^{-1}, which is higher than in the perfusion processes, such a process would require 15 d at optimal yield (Tab. 2). Thus, it would be 20 % longer, and would be much more labor-intensive due to the several process steps in shake flasks, wave-mixed and stirred bioreactors.

With minor adjustments, a 12-day inoculum production combined with a 12-day mAb production process could lead to maximum capacity utilization of the upstream section of the production plant. The N–1 bioreactor and the production bioreactor can be operated in parallel and idle times can be minimized accordingly. Since only two bioreactors would represent the entire upstream process, the plant footprint for this part of the process would be very small compared to the classical approach with an upstream process including several bioreactors and lasting several weeks.

To the author's knowledge, this study is the first of its kind to demonstrate upstream process intensification by perfusion-based one-step inoculum production, combined with a high-seeding approach for the production bioreactors. Inoculation with cryovials containing $260 \cdot 10^6$ cells mL^{-1} enables the production of around $180 \cdot 10^6$ cells mL^{-1} within only 7.5 d in wave-mixed bioreactors run in perfusion mode. For the first time in the research field, to the author's knowledge, this paper describes the subsequent use of such high VCDs achieved in the N–1 step for the inoculation of fed-batch production processes. Using high-seeding VCDs in fed-batch production processes can lead to time savings and higher IgG titers, and therefore to an increase of up to 50 % in manufacturing capacity. The results achieved in shake flasks and the ambr 250 modular system were successfully transferred to 50-L and 200-L single-use STRs. This lays the foundation for a functioning scaling approach for transfer to the cubic meter scale.

We would like to thank Thermo Fisher Scientific's Gibco team for providing the cells and the media, and Andry Ehrhart for his support during the experimental phase.

Sub- and Superscripts

max maximum value
start value at the start of the cultivation

Abbreviations

CHO Chinese hamster ovary
CSPR Cell-specific perfusion rate
IgG Immunoglobulin G
mAb Monoclonal antibody
UHCD Ultra-high cell density
VCD Viable cell density
WCB Working cell bank

References

[1] I. Hernandez, S. W. Bott, A. S. Patel, C. G. Wolf, A. R. Hospodar, S. Sampathkumar, W. H. Shrank, *Am. J. Manag. Care* **2018**, *24 (2)*, 109–112.

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[2] J. Müller, M. Teale, S. Steiner, S. Junne, P. Neubauer, D. Eibl, R. Eibl, in *Cell Cult. Eng. Technol.* (Ed: R. Pörtner), Springer, Cham **2021**.

[3] N. Ninomiya, S. Shirahata, H. Murakami, T. Sugahara, *Biotechnol. Bioeng.* **1991**, *38 (9)*, 1110–1113. DOI: https://doi.org/10.1002/BIT.260380920

[4] R. Heidemann, M. Mered, D. Q. Wang, B. Gardner, C. Zhang, J. Michaels, H. J. Henzler, N. Abbas, K. Konstantinov, *Cytotechnology* **2002**, *38 (1)*, 99–108. DOI: https://doi.org/10.1023/A:1021114300958

[5] B. Wright, M. Bruninghaus, M. Vrabel, J. Walther, N. Shah, S.-A. Bae, T. Johnson, J. Yin, W. Zhou, K. Konstantinov, *Bioprocess Int.* **2015**, *13 (3)*, 16–25.

[6] M.-F. Clincke, C. Mölleryd, P. K. Samani, E. Lindskog, E. Fäldt, K. Walsh, V. Chotteau, *Biotechnol. Prog.* **2013**, *29 (3)*, 768–777. DOI: https://doi.org/10.1002/BTPR.1703

[7] Y. Tao, J. Shih, M. Sinacore, T. Ryll, H. Yusuf-Makagiansar, *Biotechnol. Prog.* **2011**, *27 (3)*, 824–829. DOI: https://doi.org/10.1002/BTPR.599

[8] G. Seth, R. W. Hamilton, T. R. Stapp, L. Zheng, A. Meier, K. Petty, S. Leung, S. Chary, *Biotechnol. Bioeng.* **2013**, *110 (5)*, 1376–1385. DOI: https://doi.org/10.1002/BIT.24808

[9] https://cytiva-delivery.sitecorecontenthub.cloud/api/public/content/digi-17729-pdf (accessed on June 14, 2022)

[10] www.sigmaaldrich.com/deepweb/assets/sigmaaldrich/product/documents/304/890/seed-train-intensification-using-hcdc-and-specially-designed-expansion-medium-wp5751en-mk.pdf (accessed on June 14, 2022)

[11] J. Müller, V. Ott, D. Eibl, R. Eibl, *Processes* **2022**, *10 (5)*, 911. DOI: https://doi.org/10.3390/pr10050911

[12] M. Schulze, J. Lemke, D. Pollard, R. H. Wijffels, J. Matuszczyk, D. E. Martens, *J. Biotechnol.* **2021**, *335*, 65–75. DOI: https://doi.org/10.1016/J.JBIOTEC.2021.06.011

[13] https://assets.thermofisher.com/TFS-Assets/BPD/Application-Notes/application-note-dyna-drive-perfusion-cell-culture-applications.pdf (accessed on June 14, 2022)

[14] L. Stepper, F. A. Filser, S. Fischer, J. Schaub, I. Gorr, R. Voges, *Bioprocess Biosyst. Eng.* **2020**, 1–13. DOI: https://doi.org/10.1007/s00449-020-02337-1

[15] J. Xu, M. S. Rehmann, M. Xu, S. Zheng, C. Hill, Q. He, M. C. Borys, Z. J. Li, *Bioresour. Bioprocess.* **2020**, *7 (1)*, 17. DOI: https://doi.org/10.1186/s40643-020-00304-y

[16] M. F. Clincke, C. Mölleryd, Y. Zhang, E. Lindskog, K. Walsh, V. Chotteau, *Biotechnol. Prog.* **2013**, *29 (3)*, 754–767. DOI: https://doi.org/10.1002/btpr.1704

[17] J. Pollock, S. V. Ho, S. S. Farid, *Biotechnol. Bioeng.* **2013**, *110 (1)*, 206–219. DOI: https://doi.org/10.1002/bit.24608

[18] R. Kshirsagar, T. Ryll, *Adv. Biochem. Eng. Biotechnol.* **2018**, *165*, 51–74. DOI: https://doi.org/10.1007/10_2016_56

[19] D. Müller, L. Klein, J. Lemke, M. Schulze, T. Kruse, M. Saballus, J. Matuszczyk, M. Kampmann, G. Zijlstra, *Chem. Eng. Process.* **2022**, *171*, 108727. DOI: https://doi.org/10.1016/J.CEP.2021.108727

[20] I. Padawer, W. L. W. Ling, Y. Bai, *Biotechnol. Prog.* **2013**, *29 (3)*, 829–832. DOI: https://doi.org/10.1002/BTPR.1719

[21] W. C. Yang, J. Lu, C. Kwiatkowski, H. Yuan, R. Kshirsagar, T. Ryll, Y. M. Huang, *Biotechnol. Prog.* **2014**, *30 (3)*, 616–625. DOI: https://doi.org/10.1002/BTPR.1884

[22] A. Mahé, A. Martiné, S. Fagète, P.-A. Girod, *Bioprocess Biosyst. Eng.* **2022**, *45 (2)*, 297–307. DOI: https://doi.org/10.1007/s00449-021-02657-w

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