Process Intensification by Inoculating Antibody Production Bioreactors Directly from Cryovials

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Supporting Information available online

Dedicated to Prof. Dr.-Ing. Peter Czermak on the occasion of his 65th birthday

There are several ways to make antibody production processes more efficient and less expensive through process intensification. In this study, an ultra-high cell density working cell bank was used to inoculate production bioreactors directly with cells from cryovials. After lab-scale experiments, a fed-batch pilot-scale experiment was successfully conducted as a proof-of-concept. In addition, cells were cultivated in perfusion mode at 2 L scale. The intensification approach presented here allows the complete elimination of the inoculum production outside the production bioreactor and the creation of additional production capacity by saving time and space. In perfusion mode, an increase in bioreactor productivity of over 180% can be achieved compared to fed-batch mode.

Keywords: Chinese hamster ovary cells, Fed-batch, Perfusion, 1:10 Turndown ratio, Ultra-high cell density cryovial

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1 Introduction

Monoclonal antibodies (mAbs) remain the most important biopharmaceuticals, despite the approval and production boom of vaccines during the recent covid pandemic, and still account for more than 50 % of biopharmaceutical sales and newly-approved biopharmaceuticals. These antibodies are mostly produced with Chinese hamster ovary (CHO) cells and mainly used to treat inflammatory and autoimmune diseases and cancer [1].

Due to this importance for biopharmaceutical manufacturers, the improvement of mAb production processes has been the focus of attention in recent years, with the aim of reducing production costs and achieving higher bioreactor productivity and manufacturing capacities. This process intensification is applied in both the upstream and downstream operation [2]. In the upstream process, there are three areas in the focus of intensification: i. the cell bank, ii. the inoculum production, and iii. the antibody production process.

The establishment of high-cell density or high-volume cell banks in order to improve the process during cell freezing has already been described by various groups [3–11]. Here, by freezing cell densities of up to 260×10^6 cells mL^{-1} in cryovials [11] or by freezing volumes of up to 150 mL in cryobags [8], a large number of cells per frozen unit can be achieved, which in turn can make the expansion of the cells in shake flasks after thawing obsolete.

For further intensification of the seed train, N–1 perfusion is an option. Here, the last step of the seed train is carried out in perfusion mode, aiming at viable cell densities (VCDs) of up to 200×10^6 cells mL^{-1}, in order to subsequently inoculate the production bioreactor [7, 12–15]. The combination of intensified cell banking and N–1 perfusion for one-step inoculum production has already been carried out successfully [6, 10, 15].

The intensification of the mAb production process can be achieved, for example, through high-seed fed-batches, whereby the production bioreactor is inoculated with a high starting VCD to shorten the production process and achieve higher titers [16–19]. As an alternative to fed-batch processes, which continue to dominate mAb production, a trend

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towards continuous production processes has become apparent in recent years [20–26]. These perfusion processes have the advantage that the product can be harvested with a constant concentration and quality over several weeks or months [25, 27], which simplifies downstreaming. New developments in the single-use area have led to continuous production processes becoming easier and safer to execute in both the upstream and downstream areas in recent years [2]. With modern cell lines, culture media and single-use equipment, higher space-time yields can be achieved in perfusion processes compared to fed-batch processes [20, 28, 29]. Even in bioreactors that are classically called pilot-scale and not production-scale, achievable productivities > $1\,g\,L^{-1}d^{-1}$ make it possible to produce comparable quantities of antibody at the same time as in cubic meter scale single-use bioreactors run in fed-batch mode. Moreover, due to the smaller footprint, several smaller production bioreactors can run at the same time. For example, BiosanaPharma has an Omalizumab biosimilar in clinical phase III, which is produced at 50 L bioreactor scale, and which is planned as the first antibody produced in a fully continuous production process [30].

Product quality should also be considered when intensifying mAb production processes. Changes in the process can have an influence on the quality parameters. Frequently analyzed parameters are the glycosylation pattern, the ratio of charge variants of the antibody and the ratio of fragments and aggregates [31–35]. Some of these parameters are known to be critical quality attributes, i.e., they can have a major influence on biological activity, pharmacokinetics, immunogenicity, or safety. The critical quality attributes should be investigated from antibody to antibody in functional assays and clinical studies [36, 37].

In a previous study, the establishment of a cell bank with > 250×10^6 cells mL^{-1} was described [11]. This cell bank was subsequently used to successfully realize one-step inoculum production for low-seed and high-seed fed-batch processes in the lab- and pilot-scale [15]. The present study aims at further upstream process intensification by completely eliminating all seed train steps before the production bioreactor. For this purpose, fed-batch experiments as well as a perfusion bioreactor were directly inoculated with cells from an ultra-high cell density working cell bank (UHCD-WCB) cryovial (Fig. 1). At pilot-scale, the high turndown ratio of the HyPerforma DynaDrive S.U.B. bioreactor (Thermo Scientific) was used to inoculate with only 5 L working volume in the 50 L bioreactor. The approach can enable a significant simplification of the production process, as infrastructure and bioreactors would no longer be required for inoculum production and manual handling can be reduced to a minimum.

2 Material and Methods

2.1 Cell Line and Media

In all cultivations, an Immunoglobulin G (IgG)-producing ExpiCHO-S cell line (Gibco) was used. For fed-batch cultivations, Efficient-Pro Medium (Gibco) was used as basal medium and Efficient-Pro Feed 2 (Gibco) as feed solution. The basal medium was supplemented with 6 mmol L^{-1} L-glutamine (Sigma-Aldrich) and 0.1 % Anti-Clumping Agent (Gibco). High-Intensity Perfusion CHO Medium (Gibco) supplemented with 4 mmol L^{-1} L-glutamine and 0.1% Anti-Clumping Agent was used for perfusion cultivations. 0.66x concentrated medium was used at the beginning of the cultivation, 1x concentrated medium was used for perfusion.

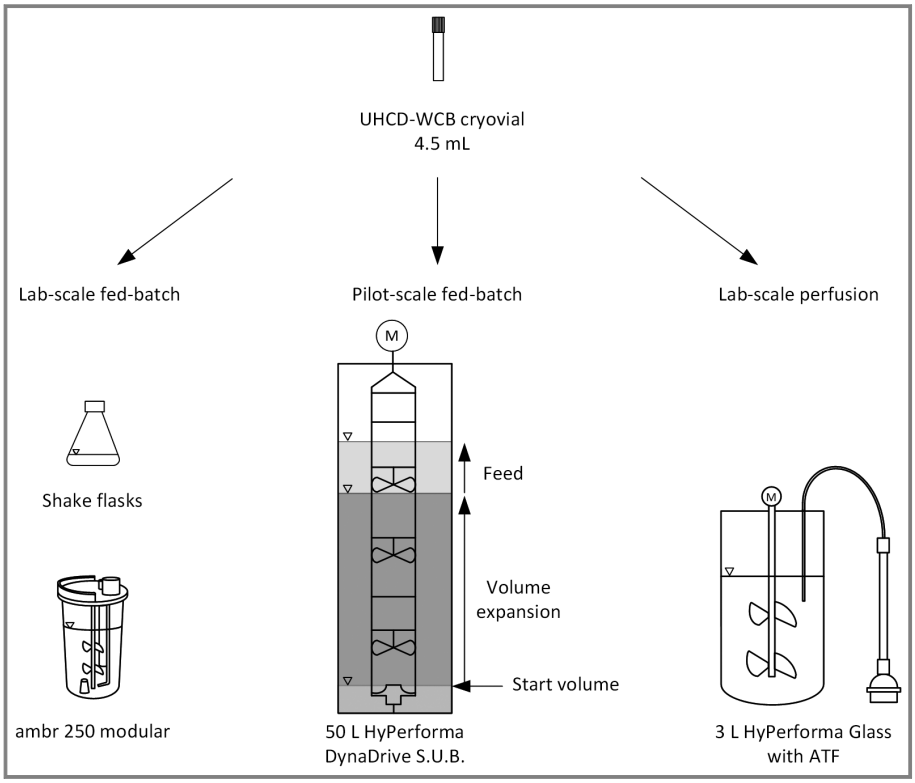

Figure 1. Schematic representation of the experiments performed with the UHCD-WCB.

2.2 Inoculum Production

The standard inoculum production was performed in shake flasks (Corning) over a period of 7 d according to the procedure described by Müller et al. [15]. In the experiments without seed train, the cryopreserved cells from the UHCD-WCB with a VCD of 260×10^6 cells mL^{-1} were thawed and either directly transferred into the cultivation systems (50 L cultivation, using a transfer bottle with pre-warmed medium) or diluted in a shake flask 1:10 with pre-warmed medium (shake flasks and lab-scale bioreactors). After the determination of the VCD, the necessary amount of cell suspension was transferred into the corresponding cultivation system.

2.3 IgG Production in Fed-Batch Mode

IgG production experiments in fed-batch mode were performed in three different cultivation systems: (i) 250 mL single-use shake flasks (Corning) with a working volume of maximum 54 mL, (ii) Ambr 250 modular vessels (mammalian version, two 3-blade segment stirrers, Sartorius) with a working volume of maximum 250 mL and (iii) the 50 L HyPerforma DynaDrive S.U.B. (Thermo Scientific), with a ladder-like stirrer assembly with three 2-pitched-blade stirrers and one 2-blade sweep stirrer, and a maximum working volume of 50 L (Fig. 1).

In the first stage, a comparison between fed-batch cultivations with standard inoculum production (shake flask) and direct inoculation with cryopreserved cells of an UHCD-WCB (shake flask and Ambr 250) was performed. For these fed-batch experiments, a seeding cell density of $0.2–0.3 \times 10^6$ cells mL^{-1} was used. After an initial batch phase of 3 d for the standard inoculum production experiments and 4 d after directly transferring the cryopreserved cells into the cultivation system, the daily feeding procedure was started. 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. To keep the glucose concentration in the cultivation systems above 2 g L^{-1}, 450 g L^{-1} glucose solution was added when needed.

In the second stage, the intensified fed-batch cultivation procedure, where no standard inoculum production was performed, was transferred to 50 L pilot-scale. Here, one 4.5 mL cryovial of the UHCD-WCB was thawed and the cells were transferred into the DynaDrive bioreactor with 5 L starting volume. According to the growth of the cells, the working volume was increased with basal medium to 38 L between day 2 and day 6 linearly to reach the start volume for the fed-batch process. As scale-down comparison at lab scale, an Ambr vessel was started in parallel with the same inoculum and the same dilution was realized daily to simulate the volume expansion, which means that the Ambr was started with the minimum working volume of 100 mL and parts of the cell suspension had to be removed from the bioreactor on the first dilution days in order to achieve the same dilution rates as in the DynaDrive. Starting on day 7, the daily bolus feeding with 2 vol % of the working volume over 12 days was carried out.

Shake flasks were incubated in a shaking incubator at 37 °C, 8 % CO_2, 80 % relative humidity and a shaking speed of 150 min^{-1} (50 mm shaking amplitude). The cultivations in the Ambr and the DynaDrive were performed with a constant specific power input of 40 W m^{-3}, 37 °C, 0.1 vvm overlay gassing, pH $\leq$ 7.2 and dissolved oxygen (DO) $\geq$ 40 %. The pH and DO setpoints were controlled either via sparging CO_2 or O_2. An 1:10 diluted Antifoam C (Sigma-Aldrich) solution was added automatically in both systems. The DynaDrive bioreactor was controlled with a G3Pro controller (Thermo Scientific).

2.4 IgG Production in Perfusion Mode

The cultivation in perfusion mode was performed in a 3 L HyPerforma Glass Bioreactor (Thermo Scientific) equipped with two 3-blade segment stirrers. As control unit, a G3Lab controller (Thermo Scientific) was used. The cultivation took place at 37 °C, 0.1 vvm overlay gassing and a constant specific power input of 165 W m^{-3}. The pH of $\leq$ 7.2 and DO of $\geq$ 40 % were controlled via adding either CO_2 or O_2 through the sparger. The bioreactor was inoculated with cells from one 4.5 mL UHCD-WCB cryovial. The perfusion mode was started on day 2 of the cultivation. According to the cell density, the perfusion rate was adjusted once daily, to keep the cell-specific perfusion rate above 55 pL $cell^{-1}d^{-1}$ until a perfusion rate of 1.0 d^{-1} was reached. It was kept constant over a period of 23 d. Subsequently, the perfusion rate was increased for the next 12 d to 1.5 d^{-1} and for the last 18 d to 2.0 d^{-1}. To keep the bioreactor volume constant at 2 L, the harvest pump was controlled by the bioreactor weight. Cell retention was realized by a suATF2 (Repligen) and the XCell Lab controller (Repligen). The average flow rate was 0.9 L min^{-1}. To keep the viable cell volume (VCV) constant at different set-points, the bleed pump was controlled with a capacitance probe (Incyte Arc, Hamilton Bonaduz). To maintain the glucose concentration at 2 g L^{-1}, a 450 g L^{-1} glucose solution was added. For this purpose, a CITSens MeMo glucose probe (Mettler Toledo GmbH, C-CIT Sensors) was used.

2.5 Analytics

Once a day, samples of the fed-batch and perfusion experiments were taken, and the cell-specific parameters VCD, total cell density, viability and cell diameter were analyzed with a Cedex HiRes analyzer (Roche Diagnostics). The concentrations of the substrates glucose and glutamine as well as the metabolites lactate and ammonium and the product IgG were determined using a Cedex Bio analyzer (Roche Diagnostics).

Antibody quality was analyzed regarding the *N*-glycosylation, the charge variants, and the ratio of low and high molecular species of the antibody. Following the purification with a Protein A resin, glycans were analyzed using hydrophilic interaction ultra-performance liquid chromatography, charge variants were analyzed using cation-exchange high-performance liquid chromatography, and low and high molecular species of the antibody were analyzed with size exclusion chromatography.

3 Results and Discussion

3.1 Fed-Batch Experiments at Lab Scale

Three fed-batch experiments each were performed in shake flasks with standard inoculum production and with direct inoculation from cryovials in shake flasks and Ambr bioreactors. Inoculation cell density was $0.20 \pm 0.02 \times 10^6$ cells mL^{-1} (SF_int) and $0.21 \pm 0.01 \times 10^6$ cells mL^{-1} (AM_int) in the intensified processes, compared to $0.28 \pm 0.03 \times 10^6$ cells mL^{-1} in the reference shake flasks (SF_sta, Fig. 2a). As already seen in our previous studies [11, 15], the growth rate on the first day in the intensified processes was 45% lower than in the reference experiments (SF_sta, 0.038 ± 0.005 h^{-1}) at 0.017 ± 0.003 h^{-1} (AM_int) and 0.017 ± 0.005 h^{-1} (SF_int), and the exponential phase was shifted by about one day. Accordingly, the feed was started on day 4 instead of day 3. The lag phase and the slight drop in viability at the start of cultivation have already been described in our previous experiments and by other groups [4, 8, 10, 11, 15].

The maximum VCD in the reference experiments was $18.5 \pm 0.9 \times 10^6$ cells mL^{-1} and was reached on day 6, in the intensified processes one day later with $16.8 \pm 1.1 \times 10^6$ cells mL^{-1} (SF_int) and $13.9 \pm 0.4 \times 10^6$ cells mL^{-1} (AM_int). Viability stayed above 90% in all experiments until the end of cultivation (Fig. 2a). That the maximum VCDs were different can be explained by the fact that the cells enter the stationary phase after four to five feed additions. If the VCD is lower at this point, it does not increase any further. The lower maximum VCD value can therefore be attributed to the previous growth. As the first feeding was started with lower VCDs ($3.4 \pm 0.4 \times 10^6$ cells mL^{-1} for SF_int and $3.2 \pm 0.3 \times 10^6$ cells mL^{-1} for AM_int compared to $4.4 \pm 0.5 \times 10^6$ cells mL^{-1} for SF_sta) and because the growth rates in the ambr experiments remained lower between day 6 and 7 (0.014 ± 0.002 h^{-1} compared to 0.019 ± 0.003 h^{-1} for SF_int), the maximum VCDs remained lower.

Nevertheless, the courses of IgG production were comparable. According to the time shift in growth, IgG production was also shifted by one day (Fig. 2b). At the time of harvest, comparable maximum IgG titers of 3.71 ± 0.14 g L^{-1} (SF_sta), 3.57 ± 0.07 g L^{-1} (SF_int), and 3.65 ± 0.03 g L^{-1} (AM_int) were achieved. These titers are similar to those described in the previous study [15]. The courses of cell diameter, glucose, lactate, glutamine and ammonium showed no relevant deviations and can be found in the Supporting Information (SI; Fig. S1). The results show that the UHCD-WCB can eliminate the need for inoculum production for lab-scale fed-batch processes.

The antibody quality was analyzed with regard to various attributes. The ratio of G0F glycans in *N*-glycosylation was between 82 ± 1% (SF_int and AM_int) and 84 ± 2% (SF_sta), the second highest ratio was G1F (SI Fig. S4a). This glycosylation pattern is promising, since human IgG is present in vivo mainly as G0F, G1F and G2F [34]. The

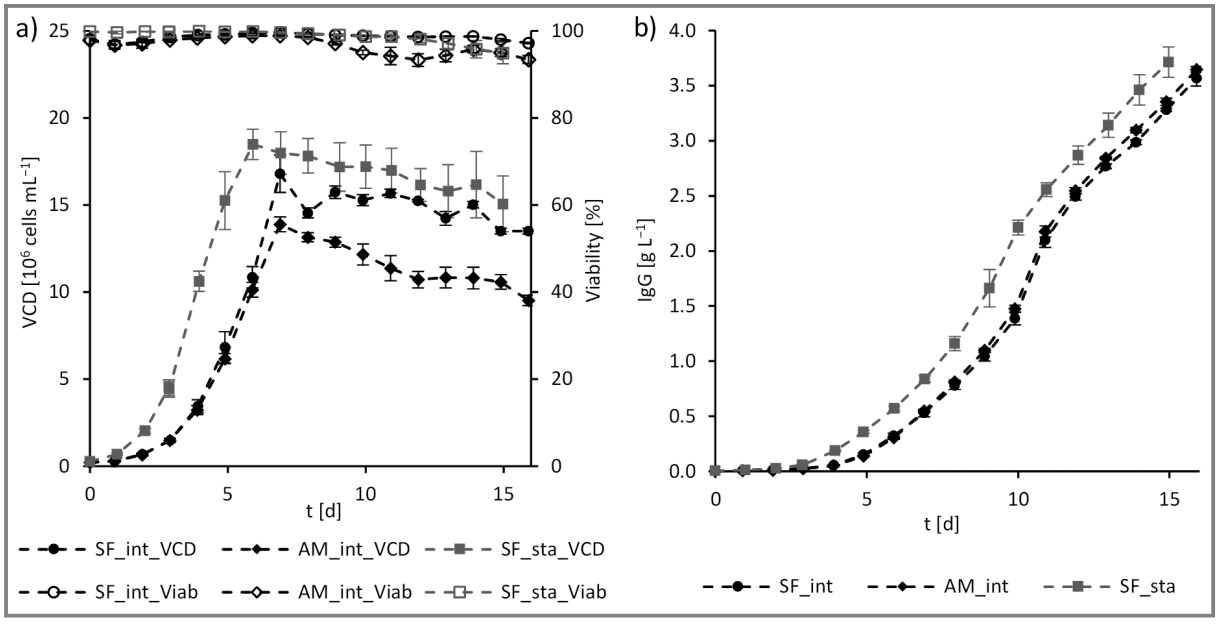

Figure 2. Time course of a) VCD and viability (Viab), and b) IgG concentration during the fed-batch cultivations with standard (sta) and intensified (int) inoculum production in shake flasks (SF) and Ambr (AM). N = 3 for all experiments.

charge variants were also similar in all experiments, with around 40 % for the main peak and around 30% each for the acidic and basic variants (SI Fig. S4b). The ratio of fragments and aggregates was low, the monomer ratio was between 93 ± 1 % (SF_sta) and 94 ± 1 % (SF_int and AM_int) (SI Fig. S4c). These results show that the use of the UHCD-WCB had no influence on the antibody quality parameters.

3.2 Proof-of-Concept Pilot Scale Fed-Batch

More interesting for the industry, however, are of course the possibilities that the UHCD-WCB opens at larger scale. Because the bioreactor concept of the DynaDrive makes it possible to work with only 5 L starting volume, the 50 L bioreactor was started with 0.18×10^6 cells mL^{-1} using only one 4.5 mL cryovial for inoculation (see Sect. 2.3). Due to the continuous dilution between day 2 and day 6 (Fig. 3b), the VCD was kept below 1.0×10^6 cells mL^{-1} until day 4 and below 3.0×10^6 cells mL^{-1} until day 6, both in the DynaDrive and in the parallel Ambr (Fig. 3a). The growth during expansion in the bioreactors was exponential after a lag phase on the first day until the first feeding on day 7. After reaching a maximum VCD of 17.0×10^6 cells mL^{-1} (Ambr: 14.7×10^6 cells mL^{-1}) on day 11, viability in the DynaDrive slowly decreased to 85 % by the end of cultivation, while in the Ambr, cells still had over 95% viability at harvest time (Fig. 3a). Nevertheless, VCD in the DynaDrive remained higher than in the Ambr throughout the entire fed-batch phase. Lower viability is most likely because in the Ambr, some of the dead cells were floated out of the suspension by foam and adhered to the bioreactor wall and other bioreactor elements, where they remained due to the almost constant working volume (Fig. 3b). Cells that adhered to the bioreactor wall in the DynaDrive, on the other hand, were suspended again by the increasing reactor volume and were accordingly also detected during sampling. The growth slowdown after day 8 in both bioreactors is atypical compared to the other experiments and led to the comparatively low maximum VCDs.

The daily specific IgG production rates q_{IgG} in the two systems were comparable (Fig. 4b) during the feeding phase (from day 7 until day 19), 22.2 ± 5.2 pg cell^{-1} d^{-1} (DynaDrive) and 22.0 ± 6.2 pg cell^{-1} d^{-1} (Ambr), and similar IgG concentrations were achieved. After the 19-day experiments, 3.58 g L^{-1} IgG was harvested in the DynaDrive and 3.49 g L^{-1} in the Ambr (Fig. 4a), comparable to the results of the standard fed-batch in section 3.1 and to previous results [15]. The courses of cell diameter, glucose, lactate, glutamine, and ammonium showed no relevant deviations and can be found in the Supporting Information (Fig. S2). The antibody quality at the end of the two fed-batch processes was comparable in all attributes with the experiments in Sect. 3.1, and no influence of the adapted process could be determined (SI Fig. S4). These results show that, taken together, a UHCD-WCB and a bioreactor with a high turndown ratio allow for the elimination of all bioreactors smaller than 50 L, saving space, time, and labor.

3.3 Proof-of-Concept 2 L Perfusion

Inoculation with cells from UHCD-WCB cryovials showed good results in fed-batch production experiments, however the trend in mAb production is increasingly towards perfu-

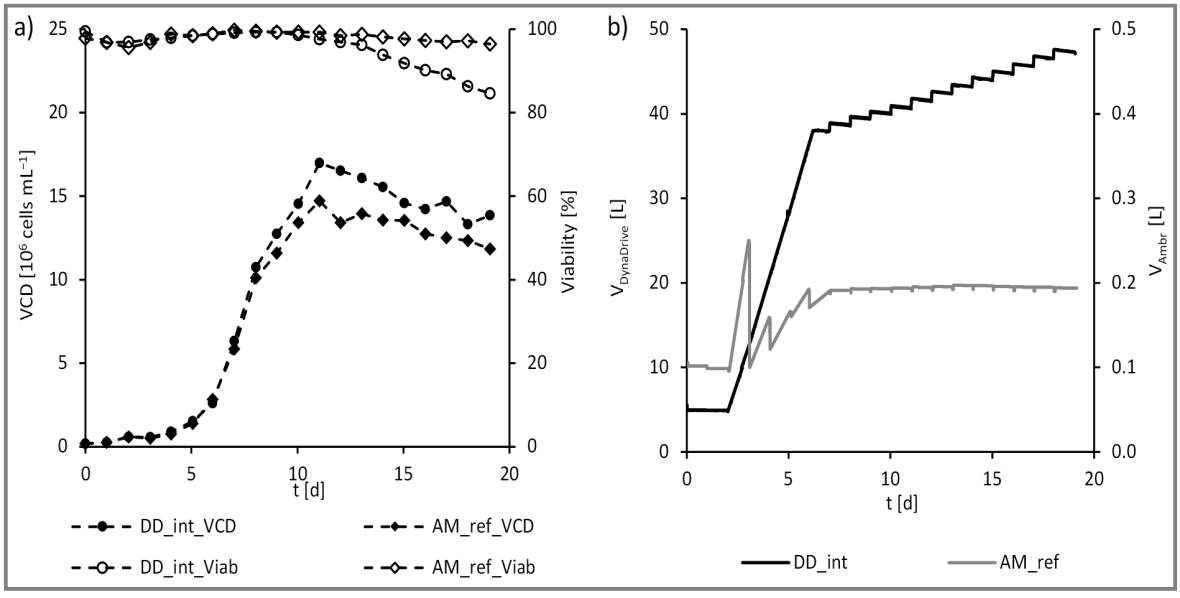

Figure 3. Time course of a) VCD and viability (Viab), and b) bioreactor volume during the fed-batch cultivations with intensified (int) inoculum production in DynaDrive (DD) and Ambr (AM) as scale-down reference (ref). N = 1 for both experiments.

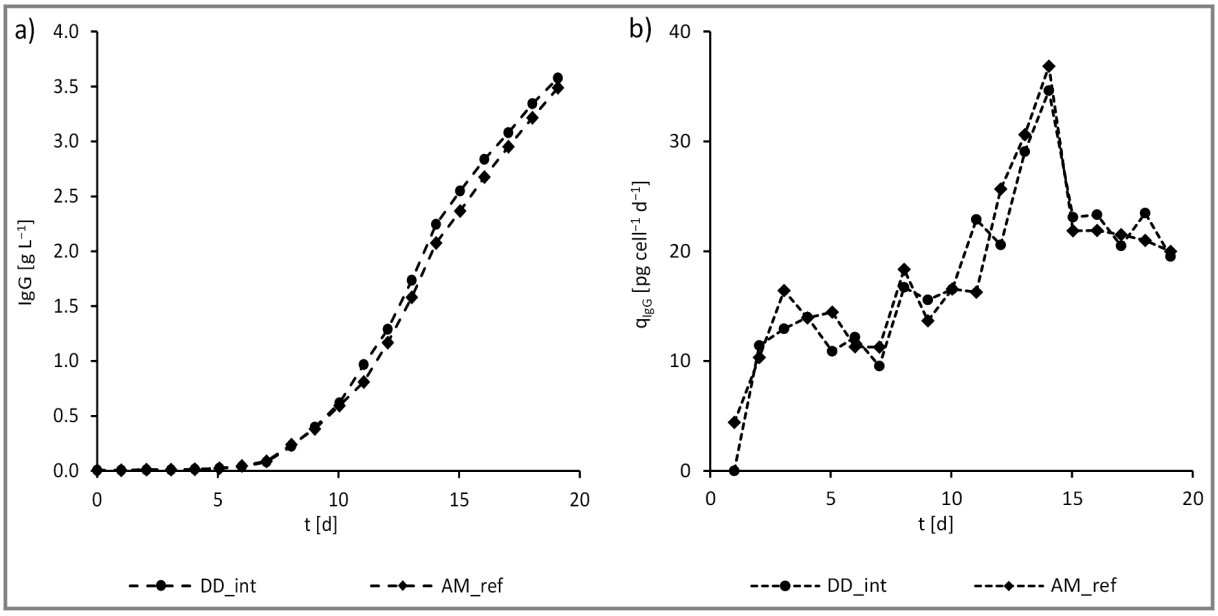

Figure 4. Time course of a) IgG concentration, and b) specific IgG production rate q_{IgG} during the fed-batch cultivations with intensified (int) inoculum production in DynaDrive (DD) and Ambr (AM) as scale-down reference (ref). N = 1 for all experiments.

sion. Therefore, a 2 L bioreactor was inoculated with cells from one cryovial and operated with a suATF2 in perfusion mode. Four different settings regarding VCV and perfusion rate were tested over a cultivation period of 57 d to determine the range for the VCV-specific perfusion rate where the VCV-specific antibody production rate and the volumetric productivity are highest and where the viability of the cells is not decreasing too fast (Tab. 1, Fig. 5a).

While the viability of the cells was above 97 % when a VCV-specific perfusion rate of 31 µL mm^{-3}d^{-1} was provided during the first phase and 41 µL mm^{-3}d^{-1} during the last phase, the viability successively decreased during phases 2 and 3, with 22 and 24 µL mm^{-3}d^{-1}, respectively, to a minimum of 87 % on day 40 (Fig. 5b).

As viability decreased, so did cell growth and, accordingly, the bleed rate, and vice versa (Fig. 6). In the two phases of high viability, the bleed rate was about 0.50 d^{-1}, between day 21 and 27 only 0.21 ± 0.07 d^{-1} and between day 29 and 38 even only 0.15 ± 0.06 d^{-1}. In order to lose as little product as possible via bleeding, the aim is always to keep cell growth as low as possible [38]. At the same time, however, the viability should not drop too far, as this can reduce the mAb quality and increase the accumulation of cell debris and also proteins, which are retained to a certain extent by the ATF. The results of this study regarding the courses of bleed rate and viability suggest that the optimal VCV-specific perfusion rate for this cell line and medium is between 25 and 30 µL mm^{-3}d^{-1}.

These results are also supported by the product formation. The highest IgG concentrations in the bioreactor and in the harvest were reached in the phase between day 21 and 27 (Fig. 7a). Subsequent increases in VCV setpoint and perfusion rate again resulted in lower IgG concentrations, but volumetric productivity, calculated from the amount of IgG harvested downstream of the ATF relative to the bioreactor volume, increased from 0.50 ± 0.07 g L^{-1}d^{-1} (d21–d27) to 0.73 ± 0.05 g L^{-1}d^{-1} (d29–d40) (Fig. 7b). In contrast, in the phases with higher viability and growth, only 0.19 ± 0.04 g L^{-1}d^{-1} (d6–d18) and 0.29 ± 0.07 g L^{-1}d^{-1} (d50–d57) could be harvested. This was not only due to the higher bleed rate, but also to the lower IgG product formation rates q_{IgG} of the cells in this phase (Fig. 7b). The volumetric productivity is a key parameter for perfusion processes. Modern processes achieve productivities of around 1 g L^{-1}d^{-1} at perfusion rates of 1.0–1.3 d^{-1} [22, 23]. However, CHO K1 cells are used in these processes, which can grow up to more than 100 mm^{3}mL^{-1} VCV at such low perfusion rates, so that comparable VCV-specific production rates like in this study are sufficient to achieve significantly higher productivities than with the CHO-S cells used in this work.

The courses of cell diameter, glucose, lactate, glutamine, and ammonium can

Table 1. Perfusion rate, targeted VCV and achieved VCV.

Cultivation time	Perfusion rate [d^{-1}]	Targeted VCV [mm^{3}mL^{-1}]	Achieved VCV [mm^{3}mL^{-1}]	VCV-specific perfusion rate [µL mm^{3}d^{-1}]
Day 6 – day 18	1.0	30	32 ± 2	31
Day 21 – day 27	1.0	45	45 ± 2	22
Day 29 – day 40	1.5	60	63 ± 4	24
Day 50 – day 57	2.0	50	49 ± 2	41

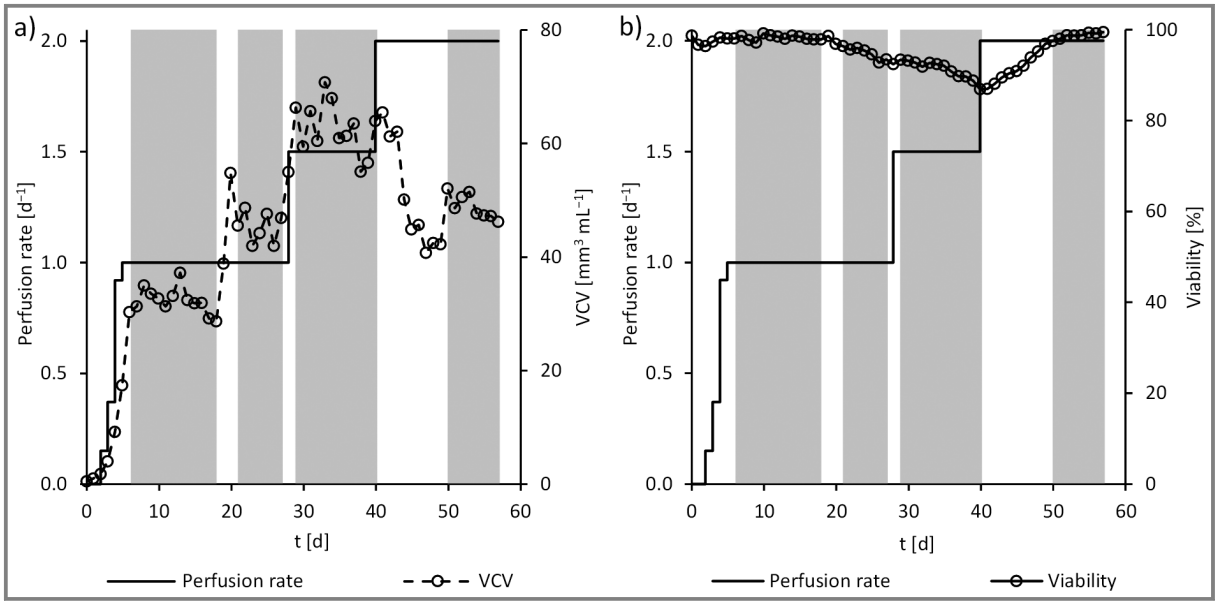

Figure 5. Time course of a) perfusion rate and VCD, and b) perfusion rate and viability during the 2 L perfusion cultivation (N = 1).

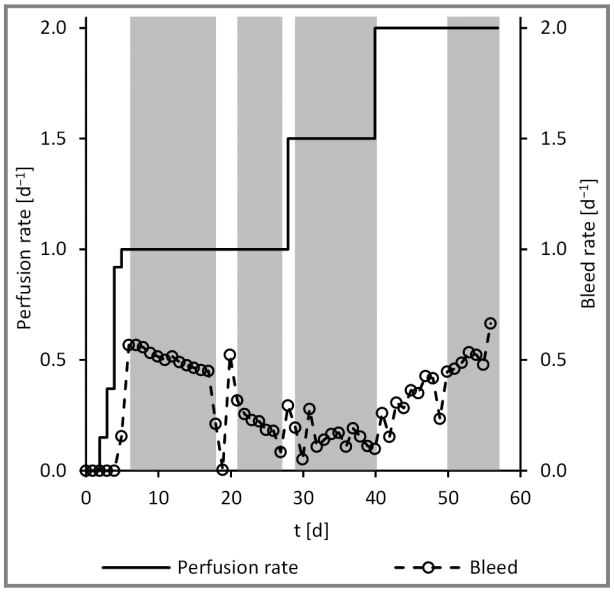

Figure 6. Time course of perfusion rate and bleed rate during the 2 L perfusion cultivation (N = 1).

be found in the Supporting Information (Fig. S3). The antibody quality was analyzed on one day during each of the four targeted steady-states (day 15, day 25, day 35, and day 55). Although the glycosylation pattern and the distribution of the batch variants was different compared to the fed-batch experiments, all quality attributes remained constant during the perfusion experiment, indicating a constant product quality, despite the different parameters set during the process (SI Fig. S5).

4 Conclusions

After in previous studies the growth and production behavior of the established UHCD-WCB in batch mode was investigated [11] as well as a one-step inoculum production was realized [15], the results achieved in this study show that, and how, the inoculum production outside the production bioreactor can be completely eliminated. In fed-batch experiments at lab-scale, the processes inoculated directly from the UHCD-WCB cryovial differed from the standard processes only by the one-day lag phase. Both growth and production were subsequently comparable (Sect. 3.1).

For industrial production processes, the promising results at pilot-scale are of much greater interest (Sect. 3.2). The successful inoculation of the DynaDrive at only 5 L working volume with one cryovial shows on the one hand that the use of the UHCD-WCB and the expansion of the cells directly in the production bioreactor can replace the 10-day standard inoculum production with only four additional expansion days, according to a time saving of 60 %, and on the other hand, reveal two possibilities for fed-batch processes at cubic meter scale: (i) A 50 L bioreactor can be inoculated with one vial, which can be operated as N–1 step in batch or perfusion mode and can be used as inoculum for low- or high-seed fed-batch cultivations at single-use production scale, even up to the two-digit cubic meter scale in stainless steel bioreactors; and (ii) By freezing a WCB in 150 mL cryobags in the UHCD range, the 5 m^3 DynaDrive bioreactor with a starting volume of 250 L could be successfully inoculated.

In addition, the UHCD-WCB was also used for a proof-of-concept perfusion experiment (Sect. 3.3). The results obtained suggest that optimization of the process may allow productivity of about 0.7 g L^{-1}d^{-1} IgG, based on the bio-

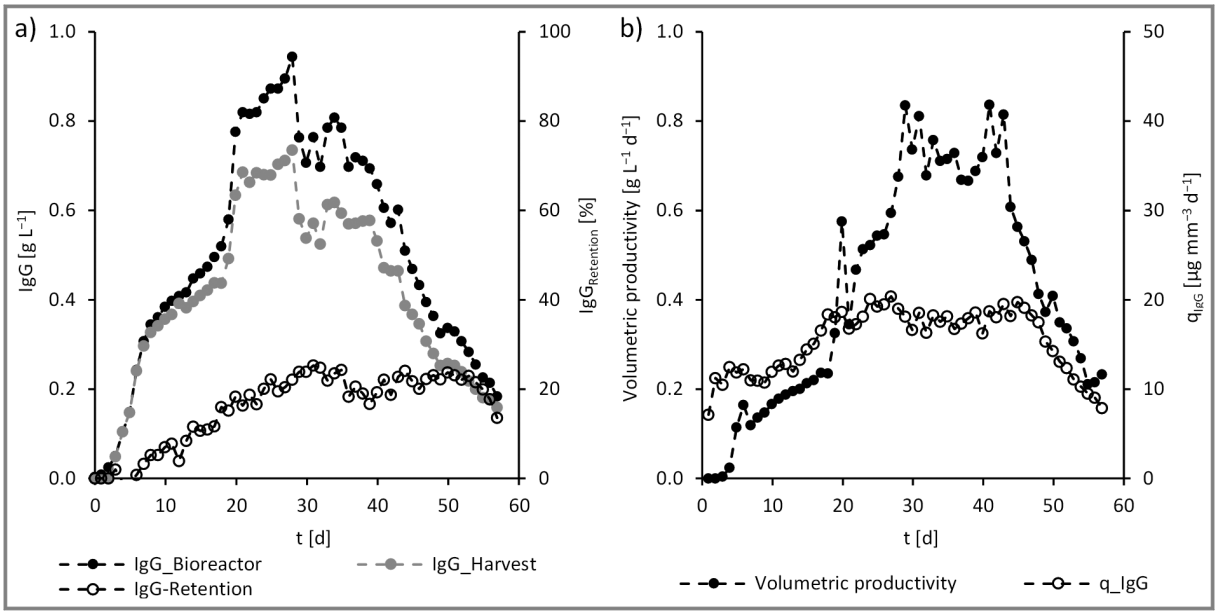

Figure 7. Time course of a) IgG concentrations in the bioreactor and the harvest and the IgG retention rate, and b) productivity and cell volume specific IgG production rate q_{IgG} during the 2 L perfusion cultivation (N = 1).

reactor volume, over a period of several weeks or months. Accordingly, excluding the unproductive growth phase at the beginning of the process, 10.5 g L^{-1} IgG could be harvested in the perfusion mode in the 15 days required for about 3.7 g L^{-1} IgG in the fed-batch mode, 180 % more. The perfusion results can be transferred to pilot and production scale analogously to the fed-batch results. Since CHO K1 cells achieve higher productivities in perfusion processes than the CHO-S cells used in this study, freezing a mAb-producing CHO K1 cell line in the UHCD range and using the DynaDrive bioreactor could realize a highly productive production process on a 50 L scale, in which no external seed train would be necessary.

To the authors' knowledge, this study is the first to demonstrate direct inoculation of production bioreactors in both fed-batch and perfusion mode with cells from cryovials. For the first time, due to the 1:10 turndown ratio of the DynaDrive bioreactor, it was also possible to inoculate a 50 L bioreactor with cells from only one 4.5 mL cryovial and perform the entire cell expansion directly in the production bioreactor. The results shown make it possible to use either exclusively production bioreactors or, if required, only additional N–1 bioreactors in production plants. Any cultivation before N–1 as well as almost all manual handling steps can be omitted, which leads to a reduction in the amount of labor, time, and space required and to a lower risk of contamination.

Supporting Information

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Symbols used

q_{IgG}	[µg $mm^{-3}d^{-1}$; pg $cell^{-1}d^{-1}$]	specific IgG production rate

Sub- and Superscripts

IgG	Immunoglobulin G

Abbreviations

CHO	Chinese hamster ovary
DO	Dissolved oxygen
IgG	Immunoglobulin G
mAb	Monoclonal antibody
UHCD	Ultra-high cell density
VCD	Viable cell density
VCV	Viable cell volume
WCB	Working cell bank

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