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RESEARCH ARTICLE

Cell Culture and Tissue Engineering

Fed-batch strategies for intensified rVSV vector production in high cell density cultures of suspension HEK293 cells

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Abstract

Vesicular stomatitis virus (VSV) has been increasingly demonstrated as a promising viral vector platform. As the interest over this modality for vaccine and gene therapy applications increases, the need for intensified processes to produce these vectors emerge. In this study, we develop fed-batch-based operations to intensify the production of a recombinant VSV-based vaccine candidate (rVSV-SARS-CoV-2) in suspension cultures of HEK293 cells. A feeding strategy, in which a commercial concentrated medium was added to cultures based on cell growth through a fixed cell specific feeding rate (CSFR), was applied for the development of two different processes using Ambr250 modular bioreactors. Cultures operated in hybrid fed-batch/perfusion (FB/P) or fed-batch (FB) were able to sustain infections performed at 8.0×10^6 cells/mL, respectively resulting in 3.9 and 5.0-fold increase in total yield (Y_T) and 1.7 and 5.6-fold increase in volumetric productivity (VP) when compared with a batch reference. A maximum viral titer of 4.5×10^{10} $TCID_{50}$/mL was reached, which is comparable or higher than other processes for VSV production in different cell lines. Overall, our study reports efficient fed-batch options to intensify the production of a rVSV-based vaccine candidate in suspension HEK293 cells.

KEYWORDS
fed-batch, HEK293 cells, high cell density, process intensification, suspension cells, viral vector

1 | INTRODUCTION

The interest for viral vectors for vaccine applications have greatly increased over the last decades. The approval and broad use of two viral vectored vaccines to fight the recent COVID-19 pandemic—ChAdOx1 (Oxford-AstraZeneca) and Jcovden (Janssen), both based on adenovirus technology—highlights the safety and responsiveness of this modality.[1,2] Vesicular stomatitis virus (VSV) is a promising vaccine platform, notably due to the low prevalence of preexisting immunity in humans and its ability to replicate to high titers in mammalian cell culture.[3–7] The approval of the VSV-based Ebola vaccine rVSV-ZEBOV (ERVEBO®) in 2019 both by the Food and Drug Administration and the European Medicines Agency underlines the potential of this platform.[8–10] Besides its application in vaccine manufacturing, the use of VSV in gene therapy or as an oncolytic virus has also shown promising results.[11,12]

Abbreviations: CSFR, cell specific feeding rate; CSVY, cell specific virus yield; hpi, hours post infection; MOI, multiplicity of infection; PR, perfusion rate; rVSV, recombinant vesicular stomatitis virus; STY, space time yield; VCD, viable cell density; VP, volumetric productivity; VSV, vesicular stomatitis virus; VVD, vessel volume per day; Y_T, total yield.

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Large scale production of rVSV-ZEBOV is conventionally performed using adherent Vero cells, currently the most accepted continuous cell line for vaccine production.[13] However, as the use of cells growing in suspension is more advantageous regarding process scale-up, the screening and development of suspension cell lines is critical. Production of recombinant VSV vectors in different suspension cell lines, including Vero cells, BHK-21, AGE1.CR, and HEK293 cells, is reported.[5,6,14–17] Of interest, comparable or even higher titers were reported for VSV production in Vero cells adapted to suspension growth, when compared with adherent Vero cells.[4,14,18] However, the high doubling time reported for the suspension-adapted cells (40–65 h, depending on the culture medium employed) limits the cell densities that can be achieved, underscoring the need for further cell line and medium development. Suspension HEK293 cells, derived from human embryonic kidney cells, are a broadly accepted expression platform for the production of recombinant proteins and viral vectors, being able to reach high cell densities in serum-free medium.[19–22] Production of VSV in suspension HEK293 cells reaching high virus titers ($0.1–1.0 \times 10^9$ $TCID_{50}$/mL for low cell density batch cultures, depending on the vector construct) has been reported,[5,6,15,17] highlighting the potential of this cell substrate.

To keep up with the demand, cell culture processes for viral vector production must be intensified. This is typically achieved by increasing the cell concentration at infection while avoiding, or at least minimizing, the so-called cell density effect, characterized by a drop in the cell specific virus yield (CSVY; virus/cell) in cultures infected at higher cell densities.[23–26] To sustain higher cell concentrations, fed-batch or perfusion operations can be used. While perfusion operations typically allow for higher cell densities and productivities with small footprints, the high volumes of medium consumed increase the final costs and the complexity of the process.[27–29] Fed-batch operations, however, balance simpler processes and reduced medium consumption with more limited cell growth and production.[27,30–33] Hybrid processes, which combine both strategies, have the potential to sustain higher cell densities and productivities while limiting medium consumption.[24,34,35]

In this study we develop efficient fed-batch-based processes to intensify the production of a rVSV-based vaccine candidate, rVSV-SARS-CoV-2, in HEK293SF cells using Ambr250 modular bioreactors. To simplify the feeding process while avoiding overfeeding, addition of concentrated feed was performed based on cell growth through a cell specific feeding rate (CSFR; pL/cell/day), defined based on the specific nutrient requirements of the cell line.

2 | MATERIALS AND METHODS

2.1 | Cell line and virus

HEK293 clone 293SF-3F6,[36] here referred as HEK293SF cells, were kindly provided by the National Research Council Canada and were cultivated in serum-free medium HyClone HyCell TransFx-H (Cytiva Life Sciences, USA) supplemented with 4 mM L-Glutamine (Gibco, USA) and 0.1% Kolliphor 188 (Sigma-Aldrich, USA). Cells were maintained in 125 mL shake flasks (25 mL working volume) in an orbital shaker incubator at 135 rpm, 37°C, and 5% CO_2. Cell counting was performed using a Vi-Cell XR Cell Viability analyzer (Beckman Coulter, USA) and main nutrients and metabolites (glutamine, ammonium, glucose, and lactate) were quantified using a blood analyzer BioProfile® FLEX2 (Nova Biomedical, USA). Average specific consumption/production rates for main metabolites were calculated as previously described by Silva et al.[24]

The temperature restricted rVSV-vectored COVID-19 vaccine candidate rVSVInd-msp-SF-Gtc (hereafter referred as rVSV-SARS-CoV-2), described in detail by Kiesslich et al.[14] was derived from a virus stock produced in BHK-21 cells and adapted to HEK293SF cells by two consecutive passages. Due to the temperature sensitivity of this construct, the temperature was shifted to 31°C after infection for all the strategies tested. Infections were performed with a multiplicity of infection (MOI; virus/cell) varying between 0.01 and 0.001. Samples for virus quantification were collected, centrifuged (2000g, 5 min), aliquoted, and stored at −80°C until further analysis.

2.2 | rVSV-SARS-CoV-2 production in shake flasks

Production of rVSV in shake flasks was performed in 125 mL shake flasks (25 mL working volume) using the conditions described above. To evaluate the effect of different MOIs, cultures operated in batch were infected at low cell densities, between 1.0 and 1.5×10^6 cells/mL, with MOIs of 0.001 or 0.01. After infection, the temperature was shifted to 31°C.

2.3 | rVSV-SARS-CoV-2 production in Ambr250 modular bioreactors

Fed-batch-based feeding strategies were evaluated using an Ambr250 modular bioreactor system (Sartorius Stedim, Germany). Single use 350 mL baffled vessels (250 mL working volume) equipped with two pitched-blade impellers (26 mm diameter) were used. The bioreactors were maintained at 37°C, pH 7.1, and stirring speed of 190 rpm. After infection, the temperature was controlled at 31°C. The DO was maintained at 50% air saturation by sparging air and/or pure oxygen in a cascade control, and the pH was controlled with the addition of $NaHCO_3$ (70 g/L) or CO_2 added to the headspace.

Ambr250 cultures were operated in batch (B), fed-batch (FB), and hybrid fed-batch/perfusion (FB/P). A detailed description of the feeding strategies tested is presented in Table 1. The bioreactor operated in batch was seeded at 0.3×10^6 cells/mL in fresh medium and, at around 1.5×10^6 cells/mL, infected with an MOI of 0.001 without medium exchange.

Fed-batch cultures were started at 2.3×10^6 cells/mL in fresh medium. During the growth phase, the same feeding strategy was applied for cultures operated in FB/P and FB: addition of commercial concentrated serum-free feed HyClone Cell Boost™ 5 (Cytiva Life

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TABLE 1 Summary of culture conditions and results for rVSV-SARS-CoV-2 production in Ambr250 modular bioreactors.

Code	B	FB/P-1	FB/P-2	FB-1	FB-2
Growth phase	Batch	Fed-batch (20 pL/cell/day)	Fed-batch (20 pL/cell/day)	Fed-batch (15 pL/cell/day)	Fed-batch (20 pL/cell/day)
Infection phase	Batch	Semi-perfusion (PR 0.5 VVD)	Semi-perfusion (PR 0.5 VVD)	Fed-batch	Fed-batch
VCD_0 (10^6 cells/mL)	0.3	2.3	2.3	2.3	2.3
VCD_{TOI} (10^6 cells/mL)	1.5	10.0	8.0	8.0	8.0
CSVY ($TCID_{50}$/cell)	3896	2353	2718	4773	4074
Y_T ($TCID_{50}$)[a]	2.2×10^{12} (reference)	3.8-fold	3.9-fold	5.0-fold	4.4-fold
VP ($TCID_{50}$/L/day)[a]	1.9×10^{12} (reference)	1.6-fold	1.7-fold	5.6-fold	4.7-fold
STY ($TCID_{50}$/L/day)[a]	1.9×10^{12} (reference)	3.4-fold	3.5-fold	5.6-fold	4.7-fold
Time of harvest	56 hpi	64 hpi	64 hpi	48 hpi	48 hpi
Total volume of medium[b]	$1 \times V_W$	$2 \times V_W$	$2 \times V_W$	$1 \times V_W$	$1 \times V_W$

Note: B, batch; CSFR, cell specific feeding rate; CSVY, cell specific virus yield; FB, fed-batch; FB/P, hybrid fed-batch/perfusion; PR, perfusion rate; STY, space time yield; V_W, working volume; VCD_0, VCD at inoculation; VCD_{TOI}, VCD at time of infection; VP, volumetric productivity; VVD, vessel volumes per day; Y_T, total yield. Each condition was performed as a single bioreactor run.

[a]From the second column onwards, presented values correspond to fold-changes in comparison to the low cell density batch reference (B) highlighted in gray.

[b]Basal + feed medium.

Sciences, USA) supplemented with 30 mM L-Glutamine (Gibco, USA) was performed every 12 h, based on a predefined CSFR (pL/cell/day), as presented in Table 1. The CSFR values adopted were defined based on preliminary experiments: briefly, HEK293SF cells were cultured in fed-batch mode and concentrated feed addition was performed based on the growth rate and the cell specific glutamine consumption rate while assuming that other nutrients were consumed at a similar rate, as described elsewhere.[24] Based on the volumes of feed added, an average CSFR value was calculated and applied in the present study. The volume of concentrated feed to be added at a given time (V_F) was calculated as follows:

$$V_F = X_{ave} * V_R * \mathrm{CSFR} * \Delta t,$$

$$X_{ave} = \frac{X_n}{2} * \left(e^{\mu * \Delta t} + 1\right),$$

where X_{ave} is the average cell density predicted for the given interval, V_R is the current volume in the reactor, Δt is the interval of time until the next feed, X_n is the current cell density, and μ is the cell specific growth rate calculated for the previous interval.

Following infection, different feeding regimes were adopted for FB/P and FB cultures. After infection of cultures operated in FB/P, performed between 8.0 and 10.0×10^6 cells/mL with an MOI of 0.001, a semi-perfusion phase was started. Cultures were semi-continuously harvested with a perfusion rate (PR) of 0.5 vessel volumes per day (VVD). For that, a determined volume of the culture was collected in spin flasks, centrifuged (300g, 3 min), and the supernatant was collected and sampled (permeate pool). Pelleted cells were resuspended in a corresponding volume of fresh medium and returned to the culture vessel. On the other hand, FB cultures continued to be operated in fed-batch after infection, performed at around 8.0×10^6 cells/mL. Due to the reduction in growth and metabolism caused by the temperature shift, addition of concentrated feed after infection was performed based on a fixed percentage. A volume of concentrated feed corresponding to 5% of the culture volume was added both at the time of infection and at 24 h postinfection (hpi). An MOI of 0.01 was chosen for cultures operated in FB to limit the accumulation of toxic by-products after infection by shortening the production phase. The total volume of concentrated feed added in the different cultures varied slightly depending on the condition tested, corresponding to approximately 25% of the final volume.

2.4 | Virus quantification

Infectious virus particles were quantified by $TCID_{50}$. Briefly, adherent HEK293 cells were cultured in DMEM medium (Gibco, USA) supplemented with 4 mM GlutaMAX (Gibco, USA) and 1% Pen/Strep (Gibco, USA). For the assay, 100 µL/well of cell suspension were seeded in a 96-well plate, with a seeding density of approximately 2.0×10^4 cells/well. After 24 h, wells were infected with 100 µL of 10-fold serial dilutions of infectious culture supernatant, with eight replicates per dilution. Plates were incubated at

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31°C for up to 7 days and evaluated under standard light microscope on Days 4 and 7 for the presence/absence of cytopathic effect in each well. $TCID_{50}$ titers were calculated following the Reed–Muench method.

2.5 | Productivity evaluation

The total yield of virus (Y_T) was calculated based on the $TCID_{50}$ titers, as follows:

$$Y_T = C_R * V_R + \sum C_P * V_P,$$

where C_R is the concentration of virus in the reactor at a given time, C_P is the concentration of virus in the permeate pool for a given time interval, V_R is the current culture volume, and V_P is the permeate pool volume.

The productivity of the different processes was assessed through the cell specific virus yield (CSVY; $TCID_{50}$/cell), the volumetric productivity (VP; $TCID_{50}$/L/day) and the space time yield (STY; $TCID_{50}$/L/day), calculated as follows:

$$\text{CSVY} = \frac{Y_T}{X_T * V_W},$$

$$\text{VP} = \frac{Y_T}{V_T * t_T},$$

$$\text{STY} = \frac{Y_T}{V_W * t_T},$$

where X_T is the maximum total cell density, V_W is the working volume, V_T is the total volume of medium consumed, and t_T is the total duration of the culture.

3 | RESULTS

3.1 | rVSV-SARS-CoV-2 production in shake flasks

To rapidly assess the effect of MOI on rVSV-SARS-CoV-2 production in HEK293SF cells, shake flask cultures operated in batch were infected at low cell densities, varying between 1.0 and 1.5×10^6 cells/mL, with an MOI of 0.01 or 0.001 (Figure 1). Peak virus production was achieved faster for cultures infected with a higher MOI, after 31–36 hpi for an MOI of 0.01 compared with 48–56 hpi for an MOI of 0.001 (Figure 1). However, as the infection with different MOIs did not result in meaningful differences in titer, the lower value (0.001) was chosen for the following experiments since lower MOIs are typically preferred to reduce the required volume of viral seed stock. Maximum titers between 4.5 and 5.5×10^9 $TCID_{50}$/mL were obtained for cultures infected at 1.0×10^6 cells/mL (SF1, Figure 1a) and between 0.8 and 1.1×10^{10} $TCID_{50}$/mL for cultures infected at 1.5×10^6 cells/mL (SF2, Figure 1b).

3.2 | Evaluation of fed-batch-based strategies for rVSV-SARS-CoV-2 production in Ambr250 modular bioreactors

A conventional low cell density batch process was performed as a reference using the Ambr250 modular bioreactor system. Cells were seeded at 0.3×10^6 cells/mL and, after growing exponentially to 1.5×10^6 cells/mL, were infected with an MOI of 0.001 (Figure 2a). Glutamine and glucose concentrations remained above limiting values throughout the culture indicating the absence of main nutrient limitation. Ammonium and lactate concentrations remained below levels that could be harmful for virus production,[37] respectively below 3 and 20 mM (Figure 3a,b). A maximum virus titer of 9.0×10^9 $TCID_{50}$/mL

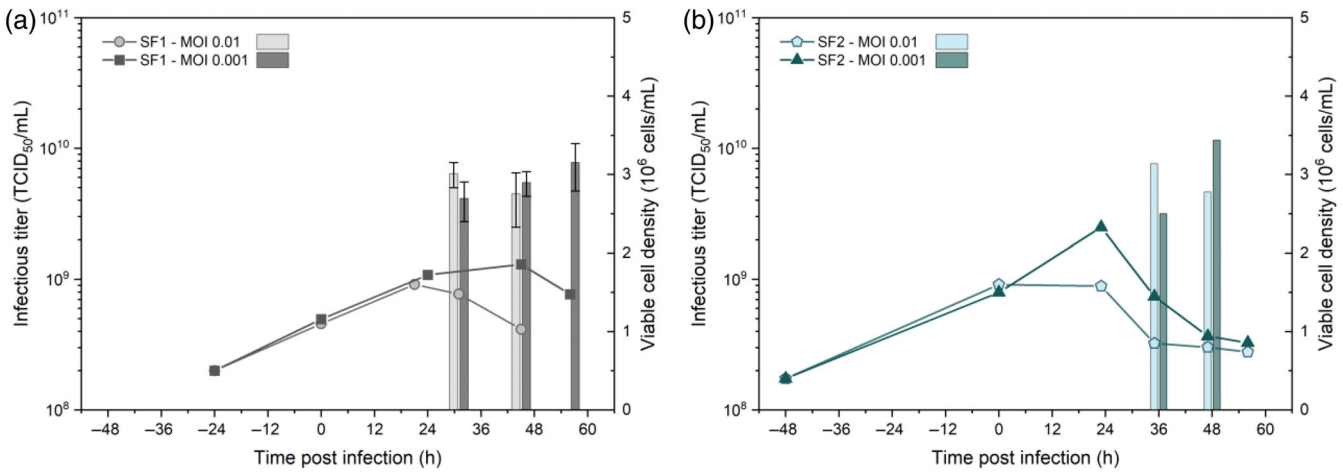


FIGURE 1 Production of rVSV-SARS-CoV-2 in suspension HEK293SF cell cultures operated in batch, performed in 125 mL shake flasks and infected with different MOIs (0.001 and 0.01). Viable cell density (symbols) and virus titer (bars). (a) SF1 cultures, infected at 1.0×10^6 cells/mL and (b) SF2 cultures, infected at 1.5×10^6 cells/mL. Cultures were performed as single replicates and error bars represent the standard deviation for technical duplicates.

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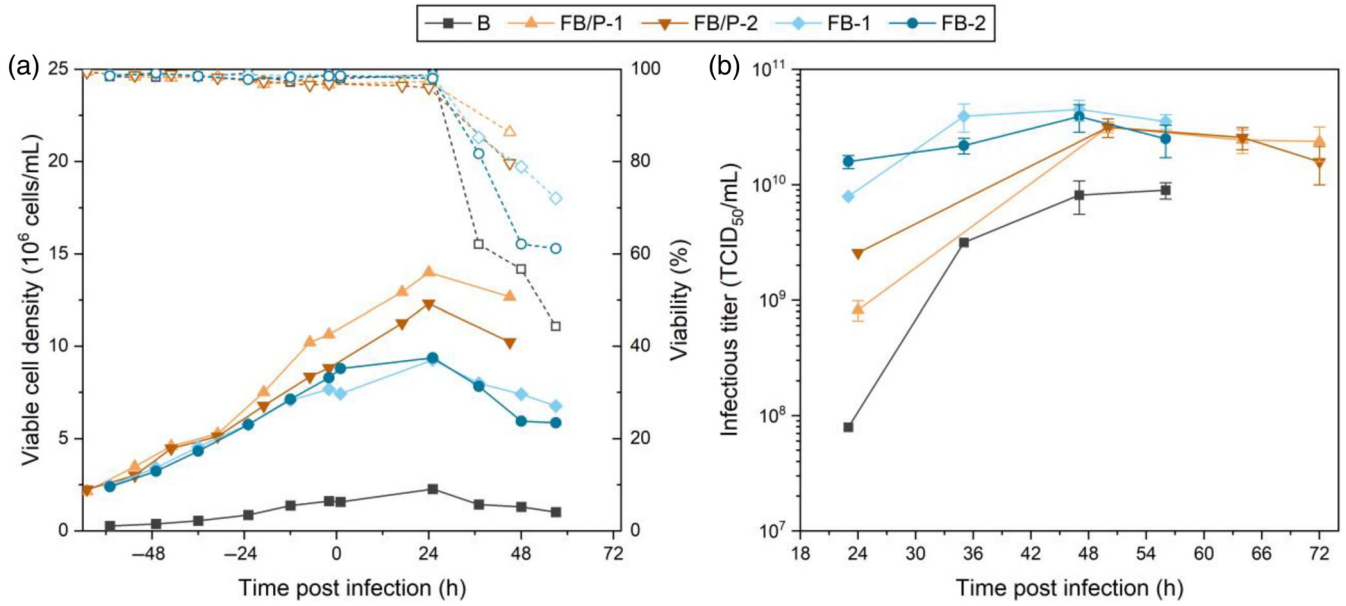


FIGURE 2 rVSV-SARS-CoV-2 production in HEK293SF cells performed in Ambr250 modular bioreactor system, operated in batch (B, infected at 1.5×10^6 cells/mL with an MOI of 0.001), hybrid fed-batch/perfusion (FB/P-1 and FB/P-2, infected between 8.0 and 10.0×10^6 cells/mL with an MOI of 0.001), and fed-batch (FB-1 and FB-2, infected at around 8.0×10^6 cells/mL with an MOI of 0.01). Each condition was performed as a single bioreactor run. (a) Viable cell density (full) and viability (empty) and (b) rVSV-SARS-CoV-2 titer (mean and standard deviation of technical duplicates).

was achieved at 56 hpi (Figure 2b) corresponding to a CSVY of 3896 $TCID_{50}$/cell (Table 1).

FB/P cultures were inoculated at 2.3×10^6 cells/mL. During the growth phase, a volume of concentrated commercial feed corresponding to a CSFR of 20 pL/cell/day was added every 12 h. After infection, performed either at 10.0×10^6 cells/mL (FB/P-1) or 8.0×10^6 cells/mL (FB/P-2) with an MOI of 0.001, a semi-perfusion operation with a PR of 0.5 VVD was initiated for continuous harvest of the produced virus. Cells reached maximal densities of 14.0×10^6 and 12.3×10^6 cells/mL at 24 hpi, respectively for conditions FB/P-1 and FB/P-2 (Figure 2a). Concentrations of glutamine and glucose did not suggest nutrient limitations during the cultivation for both conditions (Figure 3c,d). While maximum virus titers of 3.1×10^{10} $TCID_{50}$/mL were achieved at 50 hpi for both conditions (Figure 2b), maximum production was only attained at 64 hpi due to the inherent dilution effect related to the semi-perfusion operation. A CSVY of 2353 and 2718 $TCID_{50}$/cell was obtained for FB/P-1 and FB/P-2, respectively, corresponding to a decrease of up to 40% when compared with the batch reference (Table 1). This drop in CSVY indicates that some kind of cell density effect occurred for both cultures operated in FB/P and this effect seemed to be more pronounced for cells infected at 10.0×10^6 cells/mL (FB/P-1). While ammonium levels remained below 3 mM (Figure 3c), lactate concentrations of almost 40 mM were reached for condition FB/P-1 and of 25 mM for FB/P-2 (Figure 3d), which could explain, at least in part, the more pronounced decrease in specific production for the first. Nevertheless, the best performing hybrid culture (FB/P-2, infected at 8.0×10^6 cells/mL) resulted in almost 4-fold increase in Y_T (Table 1), 3.5-fold increase in STY and 1.7-fold increase in VP when compared with the batch reference (Figure 4).

Next, a conventional fed-batch process was evaluated. Cells were seeded in fresh medium at around 2.3×10^6 cells/mL. During the growth phase, in order to evaluate the effect of different CSFR values, a volume of concentrated commercial feed corresponding to 15 pL/cell/day (FB-1) or 20 pL/cell/day (FB-2) was added every 12 h. Cultures were infected without medium exchange at around 8.0×10^6 cells/mL with an MOI of 0.01, a 10-fold increase when compared with the batch reference, to evaluate if a faster production and therefore a shorter infection phase could be beneficial for VSV production at higher cell densities. Following infection, concentrated feed corresponding to 5% of the culture volume was added to both cultures at 0 and 24 hpi. Cells in both conditions, FB-1 and FB-2, reached a maximum of 9.5×10^6 cells/mL at 24 hpi (Figure 2a). Maximum virus titers of 4.5×10^{10} and 3.9×10^{10} $TCID_{50}$/mL, respectively for FB-1 and FB-2, were attained at 48 hpi (Figure 2b). Values of CSVY comparable to the batch reference were achieved for the fed-batch operations, respectively 4773 and 4074 $TCID_{50}$/cell for FB-1 and FB-2 (Table 1), demonstrating the absence of cell density effect. No meaningful difference in growth or production was observed for the two fed-batch cultivations whether feeding was performed with a CSFR of 15 or 20 pL/cell/day (Figure 2). In fact, the concentrations of main nutrients and metabolites varied slightly for both conditions but remained at very similar levels (Figure 3e,f). The comparable CSVY and higher titers obtained with the cultures operated in fed-batch resulted in up to 5-fold increase in Y_T (Table 1) and up to 5.6-fold increase in STY and VP (Figure 4) when compared with the batch reference.

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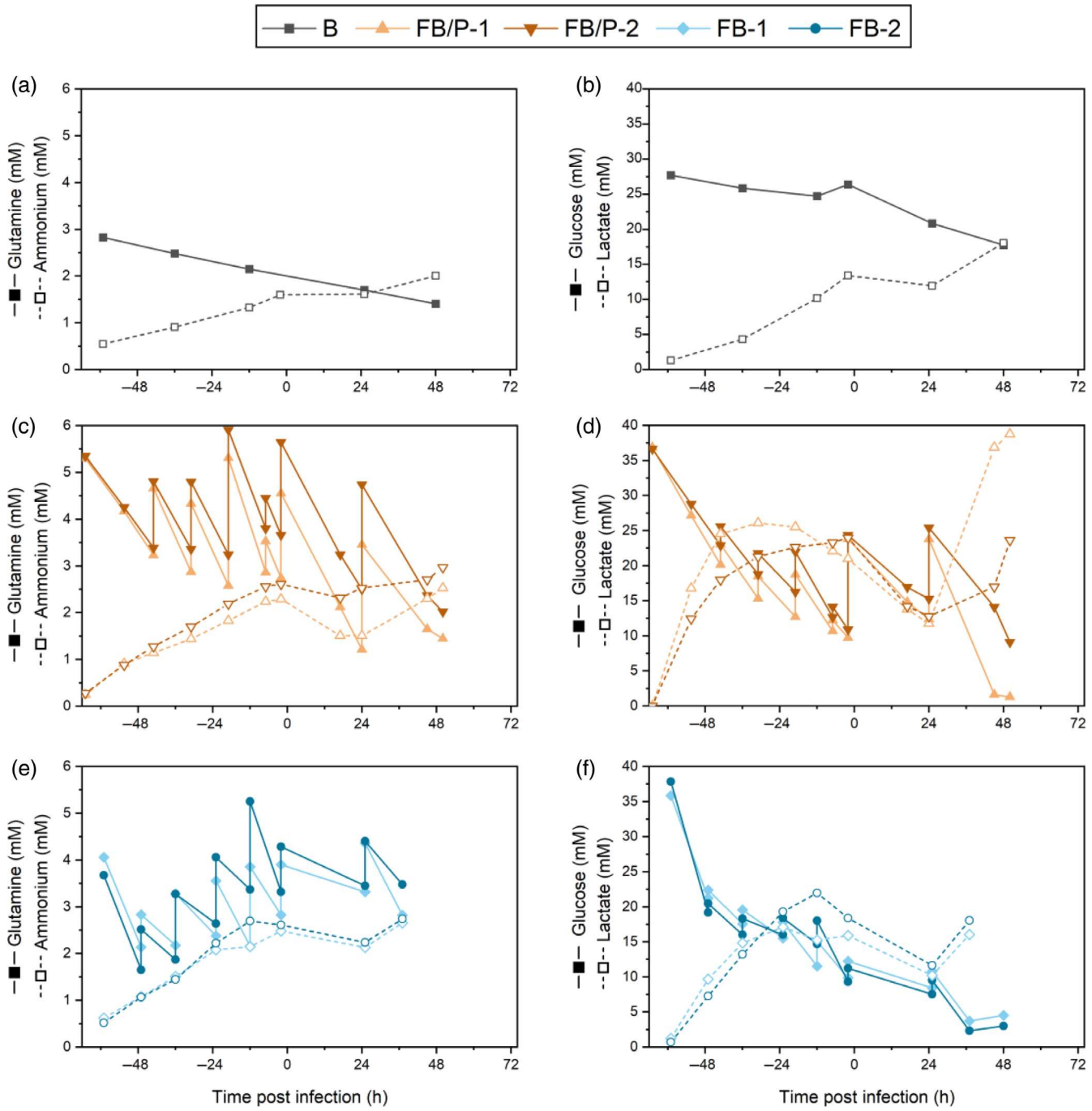


FIGURE 3 Concentration of main nutrients and metabolites in the supernatant of HEK293SF cultures producing rVSV-SARS-CoV-2 operated in batch (B, infected at 1.5×10^6 cells/mL with an MOI of 0.001), fed-batch with continuous harvest (FB/P-1 and FB/P-2, infected between 8.0 and 10.0×10^6 cells/mL with an MOI of 0.001), and fed-batch (FB-1 and FB-2, infected at 8.0×10^6 cells/mL with an MOI of 0.01). (a, c, e) concentrations of glutamine (full) and ammonium (empty) and (b, d, f) concentrations of glucose (full) and lactate (empty).

4 | DISCUSSION

4.1 | rVSV-SARS-CoV-2 production in Ambr250 bioreactors

In this study, production of rVSV-SARS-CoV-2 in a low cell density batch culture of HEK293SF cells resulted in a CSVY comparable to that attained for the same vector produced in batch cultures of suspension Vero cells (respectively 3896 and 3670 $TCID_{50}$/cell).[14] It is also worth noting that comparable virus titers were also achieved for rVSV-ZEBOV production in low cell density batch cultures of suspension HEK293SF and suspension Vero cells.[5,14] These results suggest that HEK293SF cells are a valuable alternative cell substrate for the production of VSV-based vectors. While the Vero cell line remains

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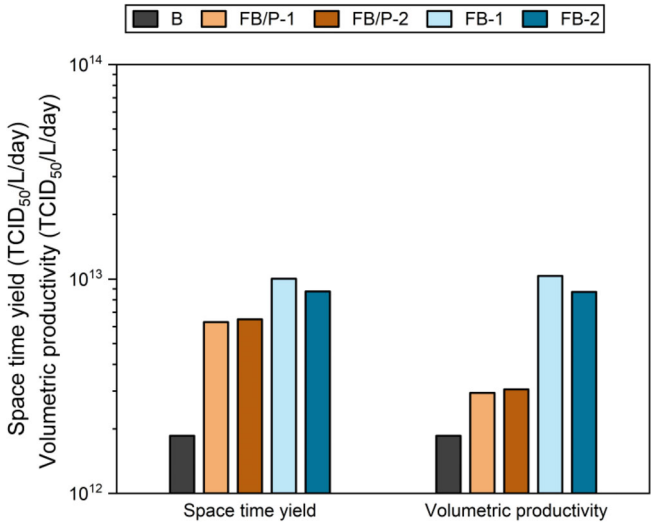


FIGURE 4 Space time yield (STY) and volumetric productivity (VP) of HEK293SF cultures producing rVSV-SARS-CoV-2 operated in batch (B, infected at 1.5×10^6 cells/mL with an MOI of 0.001), hybrid fed-batch/perfusion (FB/P-1 and FB/P-2, infected between 8.0 and 10.0×10^6 cells/mL with an MOI of 0.001), and fed-batch (FB-1 and FB-2, infected at 8.0×10^6 cells/mL with an MOI of 0.01).

the preferred host for vaccine manufacturing due to its regulatory portfolio in this type of process, an increasing number of therapeutics produced using HEK293 cells, including the COVID-19 vaccines ChAdOx1 (Oxford-AstraZeneca) and Ad5-nCov recombinant (CanSino Biologics), are being approved and arriving in the market.[2,38–40]

In a previous work, we have developed and demonstrated the efficiency of a hybrid fed-batch/perfusion process for the intensification of influenza virus production in HEK293SF cells.[24] Here, a similar process was evaluated for rVSV production. While no cell density effect was identified for influenza virus production in such hybrid mode,[24] the same was not observed here, where infections at cell densities ranging from 8.0 to 10.0×10^6 cells/mL resulted in up to 40% decrease in CSVY for FB/P cultures. The cell density effect has been often linked to nutrient limitation and/or accumulation of toxic by-products.[23–25] However, no clear limitation nor accumulation of by-products that could explain the drop in CSVY for both FB/P cultures was observed among the measured metabolite species. Using the same cell line employed in this study, Gobel et al.[17] evaluated the production of a rVSV-GFP vector in perfusion. Although no limitation of quantified nutrients (glutamine and glucose) nor accumulation of by-products (ammonium and lactate) was observed, the authors reported a 40% reduction in CSVY for a perfusion culture infected at 10.0×10^6 cells/mL when compared with a low cell density batch reference, suggesting that the observed cell density effect could be linked to other factors (e.g., metabolic stress or non-quantified inhibitors). The higher sheer stress experienced by cells in FB/P cultures during the centrifugation steps for the semi-perfusion operations could also explain the drop in cell specific virus production observed, as infected cells are reportedly more sensitive to shear stress.[41] Another possible explanation for the drop in CSVY observed for FB/P cultures in the present study is the longer production phase observed for rVSV production (up to 64 hpi) when compared with influenza (up to 48 hpi), which resulted in both cells and viruses being exposed to the harsher environmental conditions of a high cell density process for a longer period (high concentrations of lactate, ammonium, and other inhibitory compounds). While the use of higher MOIs for infection of low cell density cultures with VSV have been shown to have no or little effect on final titer[14] (Figure 1), the use of higher MOIs in high cell density processes could be beneficial to minimize the exposure of cells and viruses to the cell culture environment by reducing the duration of the infection phase. In fact, no cell density effect was observed for cultures FB-1 and FB-2—operated in fed-batch and infected at 8.0×10^6 cells/mL with a 10-fold higher MOI—despite concentrations of measurable nutrients and by-products comparable to FB/P cultures (Figure 3). These results suggest that optimal values for parameters such as the MOI, typically determined in screening studies performed in low cell density batch cultures, do not necessarily translate well when applied in high cell density processes.

No meaningful difference in cell growth and production was observed for cultures operated in fed-batch when concentrated feed addition was performed with a CSFR of 15 or 20 pL/cell/day. Both conditions sustained infections performed at 8.0×10^6 cells/mL overcoming the cell density effect. These results point out to the robustness of the developed feeding regime as small variations in the volume of added feed, as well as nutrient and metabolites concentration, did not seem to impact virus production. Elahi et al.[15] reported a fed-batch process for VSV-GFP production in high cell density cultures of HEK293SF cells, also able to overcome the cell density effect. However, the use of in-house basal and feeding medium as well as varying compositions of medium depending on the time of feed and culture phase resulted in an overall complex process. Here we propose a simpler approach, with a chemically defined commercial feed medium added based on cell growth through a fixed CSFR.

As fed-batch processes retain the produced material inside of the bioreactor until the harvest time, the stability of the virus under culture conditions, such as temperature is critical to avoid losses in infectivity in high cell density processes. Gelinas et al.[5] evaluated the stability of a rVSV vector (rVSV-ZEBOV) when exposed to various temperatures in production medium, and reported no significant difference in infectious titers for exposures of up to 48 h in temperatures ranging from 4 to 34°C. In the present study, production of rVSV-SARS-CoV-2 was performed at 31°C, as this construct was designed for replication at lower temperatures to further increase the safety of the vaccine candidate.[14] Until 48 hpi (optimal harvest time) no loss in infectivity was observed for both cultures operated in fed-batch (FB-1 and FB-2). The lower temperature employed during the virus production phase might have also contributed to the stability of the virus material produced in high cell density cultures operated in fed-batch mode.

5 | CONCLUSION

In this study, we develop and evaluate two fed-batch-based processes to intensify the production of a rVSV-vectored vaccine candidate in

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suspension HEK293SF cells using Ambr250 modular bioreactors. When applied to cultures operated in fed-batch mode, the dynamic feeding strategy developed was able to support successful rVSV production in cultures infected at 8.0×10^6 cells/mL, overcoming the cell density effect and increasing total vector production in up to 5-fold compared with a batch reference. Production of rVSV in hybrid mode, on the other hand, was affected by the cell density effect in cultures infected from 8.0 to 10.0×10^6 cells/mL, resulting in only 4-fold increase in total vector production in comparison to the same batch reference. Due to the impact of the MOI on the duration of the infection phase, our results suggest that this parameter should be reassessed under high cell density conditions when developing intensified processes for viral vector production. Overall, our results show that fed-batch processes are a simple and effective strategy to intensify viral vector production processes, notably for vectors that are stable under normal culture conditions. Moreover, we further report HEK293SF cells as a valuable cell platform for intensified production of VSV-based vectors.

AUTHOR CONTRIBUTIONS

Cristina A. T. Silva: Conceptualization; data curation; investigation; methodology; project administration; writing – original draft; writing – review and editing. **Amine A. Kamen:** Writing – review and editing; supervision; funding acquisition. **Olivier Henry:** Funding acquisition; writing – review and editing; project administration; supervision.

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CONFLICT OF INTEREST STATEMENT

The authors declare no conflict of interest.

PEER REVIEW

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DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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