Received: 18 November 2021 | Revised: 6 April 2022 | Accepted: 13 April 2022

DOI: 10.1002/bit.28117

ARTICLE

BIOTECHNOLOGY AND BIOENGINEERING WILEY

Continuous low pH viral inactivation: Operation and scaling strategy informs viral clearance study

Matthew Brown[1] | Scott Godfrey[2] | Arch Creasy[3] | Jeffery Salm[3] | Robert Fahrner[4]

[1]Boehringer Ingelheim, Process Science, Protein Science, Fremont, California, USA

[2]Boehringer Ingelheim, Manufacturing Science and Technology, Fremont, California, USA

[3]Pfizer, Bioprocess R&D, Andover, Massachusetts, USA

[4]Pfizer, Bioprocess R&D, Chesterfield, Missouri, USA

Correspondence
Matthew Brown, Boehringer Ingelheim, Process Science, Protein Science, Fremont, CA 94555, USA.
Email: brownmox@gmail.com

Abstract

A continuous viral inactivation (CVI) tubular reactor was designed for low pH viral inactivation within a continuous downstream system across multiple scales of operation. The reactors were designed to provide a minimum residence time of >60 min. The efficacy of this tubular reactor was tested with xenotropic murine leukemia virus (X-MuLV) through pulse injection experiments. It was determined that the minimum residence time of the small-scale reactor design, when operated at the target process flow rate, occurred between 63 and 67 min. Inactivation kinetics were compared between continuous operation and standard batch practices using three monoclonal antibodies. The quantification of the virus log reduction values (LRV) was similar between the two modes of operation and most of the acid-treated samples had virus concentrations below the limit of detection. However, residual infectivity was still present in the endpoint batch samples of two experiments while the continuous samples always remained below the limit of detection. This provides the foundation for leveraging a standard batch-based model to quantify the LRV for a CVI unit operation.

KEYWORDS

continuous manufacturing, Dean vortices, scale down, scale up, viral inactivation

1 | INTRODUCTION

Several biopharmaceutical companies (Coolbaugh et al., 2021; Klutz et al., 2016; Orozco et al., 2017), bioprocessing vendors (Gillespie et al., 2019; Schofield & Johnson, 2018), and academics (Kateja et al., 2021; Martins et al., 2020) have designed a low pH continuous viral inactivation (CVI) unit operation. For CVI operations that utilize an incubation chamber (e.g., tubular reactor or packed bed column), there has been a generally consistent approach to low pH CVI strategy where the product is acidified and the product's exposure to low pH is a function of the incubation chamber's volume, operational flow rate, and axial dispersion which impacts the minimum residence time (T_{min}). Within each different CVI system, the means and strategy for controlling acidification and duration can be different. Each CVI design is typically based on the interconnected strategy inherent to the specific continuous manufacturing process.

The difficulty of low pH CVI does not reside in whether enveloped viruses inactivate in acidified protein mixtures. The robustness of this concept has been repeatedly shown in batch-based models (Brorson et al., 2003; Chinniah et al., 2016), ASTM standard (ASTM, 2012), and more recently continuous operation (David et al., 2019; Gillespie et al., 2019; Martins et al., 2020). The primary challenges associated with the wide adoption of CVI are demonstrating robustness, an appropriate scaling strategy, and associated viral spiking studies.

Boehringer Ingelheim and Pfizer have codeveloped an integrated and intensified process and associated equipment called the iSKID™ system. A specific aspect of the downstream technology is the CVI unit operation. The iSKID™ system's unit operations are hydraulically linked without the use of any tanks. Each unit operation is

therefore codependent and produce operational constraints (e.g., flow rates and pressure cascade). The focus of this study was to empirically determine the minimum residence time and quantify the viral clearance capability of CVI to provide the foundation to utilize batch-based models as means to quantify viral log reduction values for a CVI unit operation in the future.

2 | METHODS AND MATERIALS

2.1 | iSKID™ CVI unit operation

By the nature of the upstream perfusion bioreactor and Protein A (ProA) affinity capture design of the iSKID™ system, the CVI unit operation required the ability to consistently and robustly acid treat ProA captured eluates coming from a wide loading density ranging from 20 to 65 g_{mAb}/L_{resin}. It is well known that the product (e.g., mAb) itself has an innate buffering capacity that results in higher acid addition requirement as the product concentration increases (Gillespie et al., 2019). In addition, affinity chromatography experiences self-sharpening peaks that can result in concentrated elution peaks. Like most companies that have defined an end-to-end continuous manufacturing process that incorporates a low pH CVI step (Coolbaugh et al., 2021; David et al., 2019, 2020), a mixing step occurs where a single ProA elution peak, which is shaped like an asymmetric Gaussian curve, is blended. This converts the feed material of the subsequent unit operations to be near homogeneous in composition and more reflective of a load material from a batch process with a pooling step.

Once the product leaves the hydraulically linked mixer, acidification buffer is added in-line through a side stream and the mixture moves through a helical static mixer. The now acidified product is pH verified by an in-line pH probe before entering the tubular reactor. The acidification step of the CVI process uses a fixed metered volumetric addition of acid independent of protein concentration. The successful operation of the acidification is accomplished by designing the acidification buffer to be strong enough to reach the target pH range for inactivation at elevated protein concentrations but weak enough to not over titrate the protein and impact product quality. This strategy does not rely on a feedback loop as a function of the measured inline pH, rather the pH probe measurement is to verify that the product has reached the target pH.

Once the product is acidified, it then travels through a 3-D printed tubular reactor to supply the duration of acid treatment. The main drivers of the contact time in the acidic condition are a function of the reactor volume, flow rate, and the axial dispersion experienced within the reactor. When designing the tubular reactors, the process scale reactors were required to meet the following criteria:

1. Minimize differential pressure across the tubular reactor.
2. Minimum residence time of >60 min.
3. Maximum residence time of <90 min.

Since the unit operations of the iSKID™ system are hydraulically connected in series, there is an inherent pressure cascade through the system and therefore all unit operations need to be optimized to decrease pressure without affecting the function of the unit operations. For the tubular reactor, it was a matter of determining the ratio of internal diameter and path length. Pressure drop across a tube increases with smaller internal diameters and longer tubing lengths. To minimize the differential pressure, the internal diameter needed to be maximized and the reactor length needed to be minimized, while also still maintaining the minimum and maximum residence time constraints. Too short of a minimum residence time would risk the viral safety of the step, while overexposure to the acidic condition could impact product quality. The 60 min minimum residence time and 90 min maximum residence time are based on the historical batch endpoints for low pH viral inactivation. Leveraging the methodologies from Brown et al. (2020) and Brown and Orozco (2021), three reactor designs were made (Table 1) such that the quantity of material that exits the reactor at the minimum and maximum residence time would be approximately <0.00003% and >99.865%, respectively. Figure 1 displays the residence time distribution of the three scales of reactors using riboflavin as a pulse injection (2.5% v/v of the reactor volume) and flushed with approximately two reactor volumes of DI water at the process flow rate.

2.2 | Mobile phases and flow chamber

The jig-in-a-box (JIB) tubular flow reactor was designed from previous development projects (Brown & Orozco, 2021; Brown et al., 2020; Orozco et al., 2017; Parker et al., 2018), and was 3D printed utilizing SLA Technology and Accura ClearVue by 3D Systems. The JIB's design is characterized by a repeating serpentine-like pattern which generates Dean vortices. The xenotropic murine leukemia virus (X-MuLV) experiments were conducted with three 0.318 cm i.d. JIB's connected in series by two three-way stopcocks for the minimum residence time and low pH inactivating studies. The stopcock allowed for the inline sampling along the path length of the reactor for the inactivation studies and was kept inline for the minimum residence time study to maintain the flow path used for the inactivation study. The sampling location between the first and second JIB will be referred to as JIB(1), sampling location between the second and third JIB will be referred to as JIB(2), and sampling location after the third JIB will be referred to as JIB(3) (Figure 2).

For each experiment, the system and JIBs were sanitized with 0.1 M NaOH, and new stopcocks were installed. The riboflavin and Tris buffer saline (TBS) used in creating the mobile phases were purchased through Thermo Fisher Scientific. The TBS was composed of 50 mM Tris with 150 mM NaCl pH 7.5. The three monoclonal antibody species used for the inactivating experiments were source from three 100 L bioreactor perfusion runs using the iSKID™ system, and the pools were generated from a single cycle of Protein A.

TABLE 1 Scaling strategy of the iSKID™ system's CVI unit operation.

Scale	Internal diameter (cm)	Average residence time (min)	Reactor volume per hour	Shortest incubation time (T_{min}) (min)	Longest incubation time (T_{max}) (min)
Scaled-down model	0.318	78.5	0.764	≥60	≤90
iSKID™System with 100 L bioreactor	0.635	78.5	0.764	≥60	≤90
iSKID™ System with 500 L bioreactor	1.50	78.5	0.764	≥60	≤90

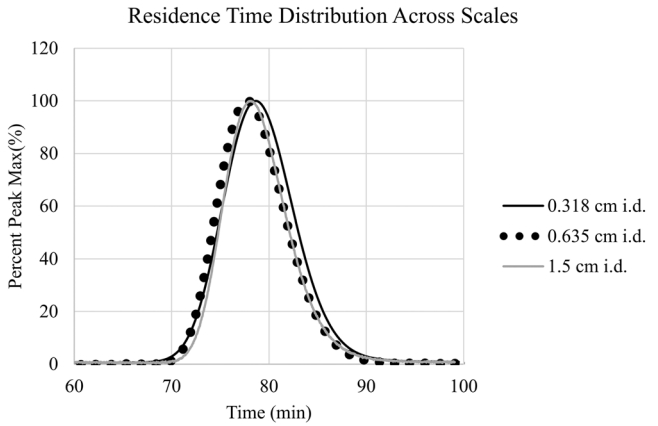

FIGURE 1 Results of pulse injections of riboflavin flushed out at the operational flowrate at three scales resulting in similar residence time distributions.

2.3 | X-MuLV quantification

X-MuLV titer was quantified by using a 50% tissue culture infectious dose (TCID50) assay as a standard service offered by Charles River Laboratories. Details regarding these assays can be found in David et al. (2019).

2.4 | Minimum residence time studies

To characterize the minimum residence time of the reactor and identify any effect of flowing through the JIBs on X-MuLV infectivity, protocols were adopted from Brown and Orozco (2021). The 0.318 cm i.d. 3D printed JIB was connected to an AKTA™ Avant 150 from Cytiva. The JIB was fully primed with TBS. A 50 ml aliquot of the TBS with 50 mg/L riboflavin was spiked at 5% v/v with X-MuLV resulting in a mobile phase concentration of ~3×10^5 virus titer/ml. The reactor was injected with the viral spiked load at 3% reactor volume by the sample pump at a flow rate of 0.764 reactor volumes per hour, and subsequently flushed with TBS at the same flow rate. An aliquot of the load material was held on the bench as a hold sample to determine if significant X-MuLV inactivation occurred as a function of mobile phase condition independent of flowing through the JIB.

After a predetermined amount of time, the outlet valve switched to direct flow into three fixed volume fractions. Once the three fractions were taken, the rest of the experiment was collected into a fourth large volume fraction. The three small volume fractions were titered via TCID50 in which the entire fraction collected was plated. These samples were intended to capture the first breakthrough of X-MuLV to quantify T_{min}. The fourth large volume fraction was titered by standard low volume TCID50. The purpose of the fourth fraction was to determine if significant X-MuLV infectivity was lost by flowing through the reactor. To prepare for the next experiment, all viral contacting flow paths of the Akta system and the CVI reactor

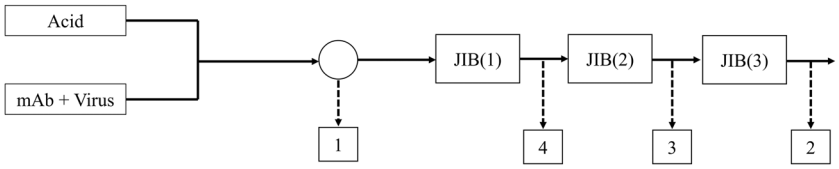

FIGURE 2 Flow schematic of the inactivation study. The numbered boxes correspond to the sampling locations in the order they were taken. Box 1 illustrates where the batch sample was created, and Boxes 2, 3, and 4 correspond to JIB(3), JIB(2), and JIB(1) samples respectively.

were sanitized with 0.1 M NaOH, and the stopcocks were replaced. After sanitization, the system was then flushed with TBS.

2.5 | Inactivation studies

At the core of low pH inactivation studies, a combination of acid, mAb, and virus spiking fluid needs to be generated. For this experimental series, a viral spiked mAb pool is acidified using in-line mixing. To avoid unintended X-MuLV inactivation as a function of the acidic nature of ProA pools, all three mAb ProA pools were neutralized to pH ~5.5 using 2 M Tris Base.

To begin an experiment, ~700 ml of mAb pool at pH 5.5 was spiked with X-MuLV at 5% v/v. An aliquot of the load material was held on the bench as a hold sample similar to what was done for the minimum residence time study. Next an aliquot of ~10 ml of viral spiked mAb pool was taken and titrated on the benchtop with the acidification buffer (i.e., 2 M glycine pH 3.3) to identify the v/v ratio of viral spiked mAb to acid required to reach a pH target of 3.60–3.65. Once this number was identified, the Unicorn method was updated to reflect the v/v ratio. The experiment itself started with first flushing the JIBs with DI water and visually inspecting the flow path for air. Once all the air was removed, 5% of the reactor volume was filled with acidification buffer at the inlet of the JIB to safeguard against any unacidified protein at the front of the load. This initial volume has no impact on the inactivation kinetics as the slug is flushed from the reactor before samples are taken.

The purpose of the inactivation studies is to compare the inactivation kinetics of a standard static hold versus CVI. The CVI reactor inherently experiences axial dispersion which creates a residence time distribution and thus leads to a distribution of acidification durations. Figure 3 displays the effect of dispersion on the residence time distributions of one JIB, two JIBs connected in series, and three JIBs connected in series (i.e., JIB(1), JIB(2), and JIB (3), respectively) by pulse injection experiments with riboflavin. These sampling points for the CVI will be matched with an equivalent batch duration as a function of the approximate T_{min} of the three reactors.

The A1 line of the Akta Avant was placed into the viral spiked mAb pool and the Akta program was initiated. The method started by first priming the lines through the injection valve at the correct % addition of acidification buffer needed to reach the target low pH of the viral spiked pool. Once primed, the injection valve switched

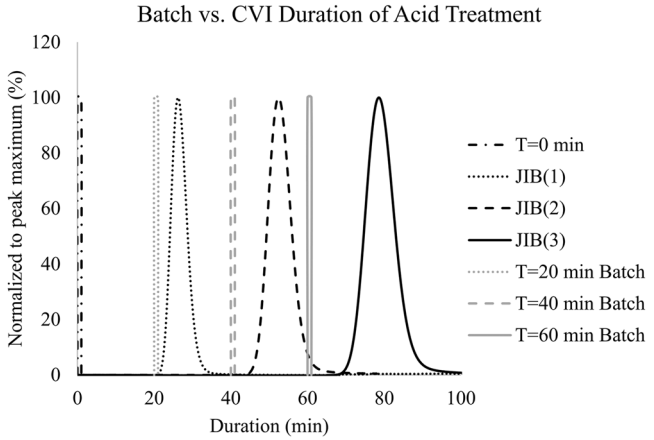

FIGURE 3 The duration of acid exposure for the tubular reactor such that JIB(1) represents one JIB, JIB(2) represents two JIBs connected in series, and JIB(3) represents three JIBs in series. Batch data points are represented by a 1 min wide bar representative of the 1 min of sample generation at $T = 0$ min. The JIB(1), JIB(2), and JIB(3) curves were generated experimentally via pulse injection experiments.

to then discharge 25 ml of acidified viral spiked pool into a tube at 25 ml/min creating the representative batch sample. This batch sample was then incubated to create the time points 0, 20, 40, and 60 min, where time point 0 min was sampled for viral titer immediately after the sample was generated. Time points 0, 20, and 40 min were analyzed by the standard TCID50 assay and the 60 min was analyzed by large volume plating (LVP) TCID50 and therefore lowered the limit of detection. After the Akta generated the batch sample, the instrument began flowing into the JIB. Figure 4 displays the resulting chromatogram which is characterized by first evacuating the reactor of the primed DI water and the breakthrough of the acid slug and viral spiked mAb followed by a steady state. Once the chromatogram displayed signals indicating steady state, the sampling could begin. The sampling for time points began at JIB(3), followed by two samples taken by three-way stopcocks (i.e., JIB(2) then JIB(1)). Samples taken at JIB(1) and JIB(2) were analyzed by the standard TCID50 assay and the sample taken at JIB(3) was analyzed by LVP TCID50. To prepare for the next experiment, all viral contacting flow paths of the Akta system and the CVI reactor were sanitized with 0.1 M NaOH, and the stopcocks were replaced. After sanitization, the system was then washed out with DI water.

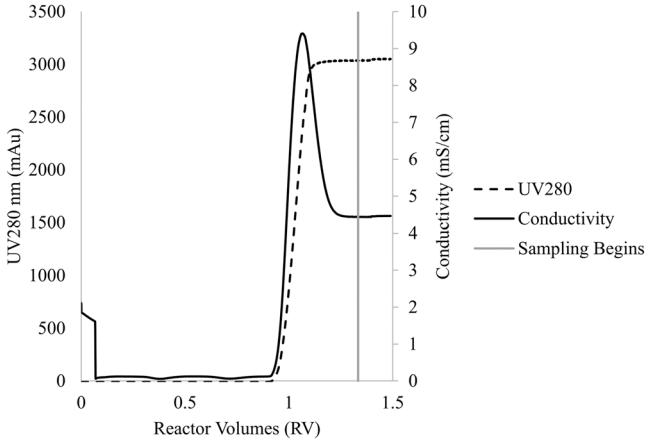

FIGURE 4 Resulting chromatogram generated by an inactivation experiment which is characterized by first voiding DI water, breakthrough of acid slug and protein, attaining a steady state, and finally beginning the sampling.

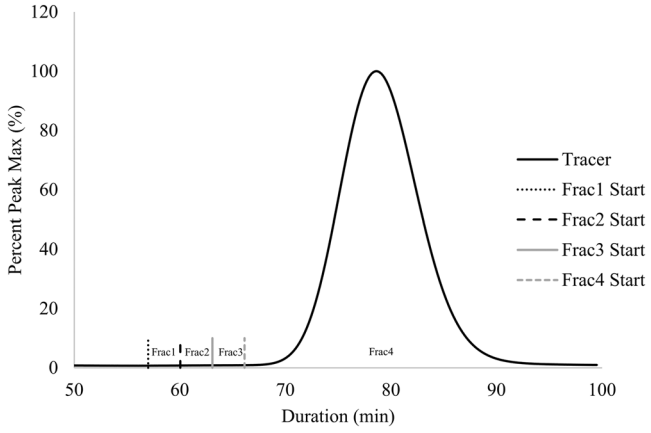

FIGURE 5 Resulting residence time distribution from the X-MuLV spiked riboflavin pulse injection experiment.

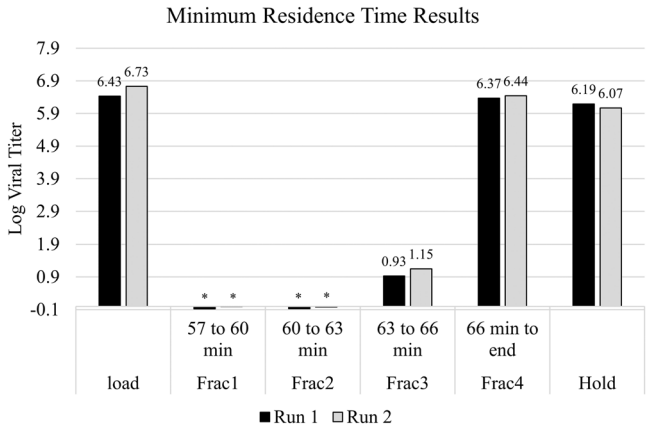

FIGURE 6 Results of the minimum residence time experiments where first breakthrough occurs between 63 and 66 min. *indicates no virus detected in the sample.

3 | RESULTS AND DISCUSSION

3.1 | Minimum residence time study

Figures 5 and 6 display the results of the pulse-chase experiments to define the minimum residence time. For the initial two small volume fractions (i.e., 57–60 and 60–63 min), no viral particles were detected. It was not until fraction 3 (i.e., 63–66 min) that X-MuLV was detected. This indicates that the T_{min} for this reactor occurs sometime between 63 and 66 min when the reactor is operated at the process flow rate. Fraction 4 collected the remaining peak traversing the JIB, and the viral load within that fraction matched the quantity injected with LRV of 0.06 and 0.29. Therefore, the JIB does not significantly inactivate or adsorb X-MuLV. Additionally, the hold sample experienced minimal loss in infectious X-MuLV (i.e., LRV of 0.24 and 0.66) and therefore the running buffer of the experiment did not significantly contribute to inactivation.

3.2 | Acidification of the mab pool

Table 2 lists the pH of the mAb pool after adding the viral spiking fluid, and the corresponding percentage of acidification buffer required to bring the viral spiked mAb pool to pH 3.60–3.65 by titrating an aliquot on the bench top. Figure 7 displays the pH of the offline titration, batch sample generation, and the three samples taken by JIB(1), JIB(2), and JIB(3). For mAb A and B's Run 1 and 2, there was good agreement between the offline, batch, and three CVI samples with the maximum difference within the individual run data set was <0.05 pH units. For mAb C, there was a noticeable difference in pH between the offline titration that determined the v/v% acid addition and the samples generated from the Akta pumps. Though the batch and CVI samples did not reach the targeted pH set point of 3.60–3.65, the experimental results of mAb C Run 2 were still valid as the pH of the batch and CVI samples were similar (i.e., a difference of <0.05 pH units across the four samples).

TABLE 2 Resulting pH of mAb pool after addition of viral spiking fluid and the percentage required to bring pH to 3.60–3.65 via offline titration.

Molecule	Postspike pH	%B
mAb A Run 1	5.63	15.4
mAb A Run 2	5.58	15.4
mAb B Run 1	6.15	19.2
mAb B Run 2	6.20	21.6
mAb C Run 1	5.86	12.6
mAb C Run 2	5.80	11.1

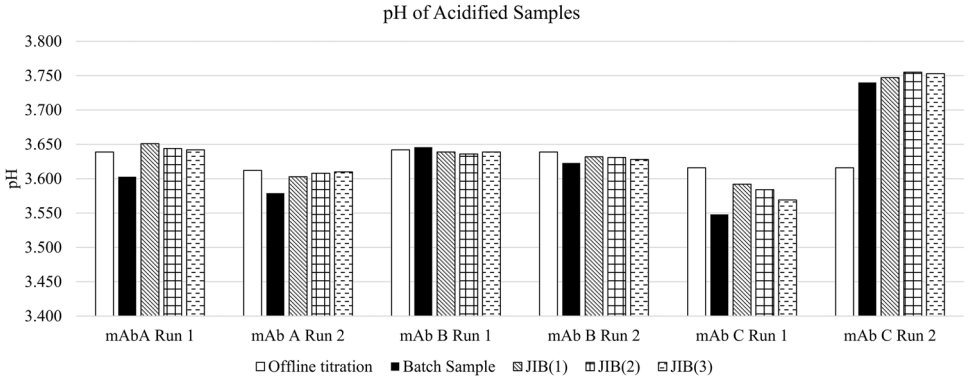

FIGURE 7 pH readings from the offline titration, batch sample generation, and the three samples taken by the two stopcocks and the out valve of the Akta.

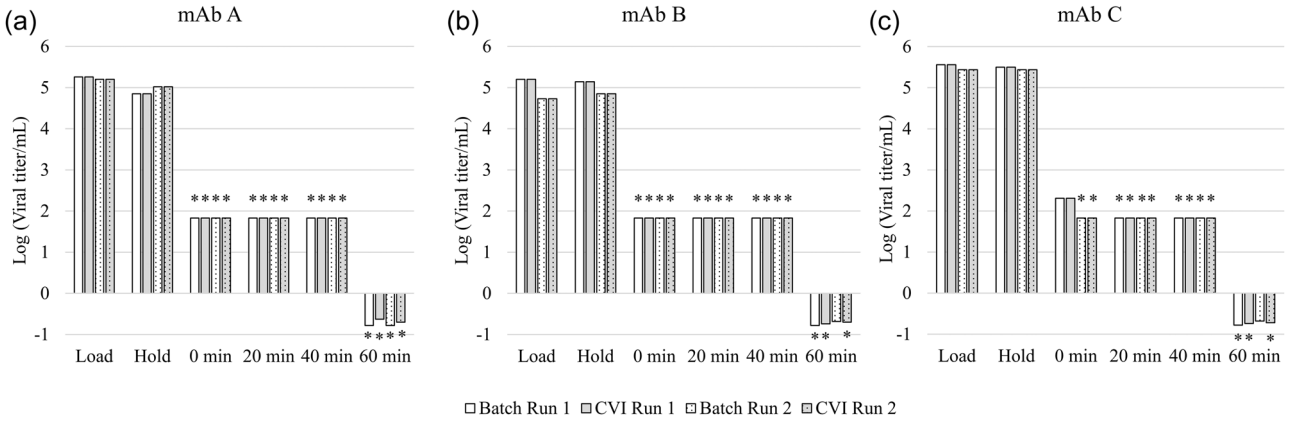

FIGURE 8 TCID50 results from the head to head batch versus CVI experiments of mAb A (a), mAb B (b), and mAb C (c). *indicates no virus detected in the sample.

3.3 | Inactivation study

Duration of low pH exposure is a critical parameter in this study. The static hold of the batch sample allows for a straightforward analysis of acid treatment duration. In the case of this study, the batch sample has a near uniform low pH time duration of 60 min. The acidified product stream flowing through the CVI has a residence time distribution due to dispersion. The endpoint sample from the CVI has an average residence time (i.e., reactor volume divided by flow rate) of 78.5 min and a minimum residence time of 63–66 min based on the noninactivating studies.

Figure 8a–c displays the results of the inactivation studies. The load, hold, and 0 min samples were identical for the batch and CVI samples for each run. The load represents the viral titer of the mAb load material immediately after the addition of the viral spiking fluid, while the hold represents a retesting of the mAb load material once the experiment was completed. For all experiments, there was a <0.5 log difference between the load and hold samples, indicating that the virus was stable in the preacidified mAb pool. The 0 min sample was generated by flowing acidified viral spiked mAb pool through the Akta's injection valve into a collection tube and immediately sampled for viral titer. For all runs, except mAb C Run 1, this sample lacked viral activity and therefore was below the limit of detection.

For the batch and CVI's 20 and 40 min samples, mAb A, B, and C lacked viral activity and therefore was below the limit of detection. The longest incubated sample of 60 min for batch and CVI was titered using LVP increasing the detection limit of the assay. For mAb A, for both batch and CVI, no viral activity was detected in the 60 min samples resulting in an average LRV of ≥6.0 and ≥5.9, respectively. For mAb B, similar LRVs were observed; however, residual viral infectivity was observed in the batch sample of Run 2. This resulted in average LRV of 5.7 for batch and ≥5.7 for CVI. The same result was observed for mAb C in that residual viral infectivity was observed in the batch Run 2 sample with the resulting average LRV of 6.2 for batch and ≥6.2 for CVI. The result of CVI containing no residual virus relative to the batch counterpart samples is expected as CVI samples have an overall longer acid exposure compared to the batch samples. This was due to the CVI sample time points being defined by minimum residence time (Figure 3) rather than their average

residence time of 78.5 min. This ensures a minimum residence time of >60 min can produce similar inactivation as a 60 min static hold with the batch being the worst case.

4 | CONCLUSIONS

This study has successfully confirmed that a tubular reactor can be designed to have a minimum residence time of >60 min. It also showed that operations in a batch mode and CVI mode yield similar inactivation kinetics; however, the exact comparison is limited as most acidified samples were below the limit of detection. It is notable that for mAb B and C, residual viral infectivity was detected in the batch sample and not for the CVI sample and therefore would be considered the worst case. Combining the results of the minimum residence time and inactivation study results, it is reasonable to propose quantifying CVI inactivation kinetics using the standard batch approach.

ACKNOWLEDGMENTS

The authors acknowledge Joelle Khouri, Debola Banerjee, and Tobias Moritz of Boehringer Ingelheim (Fremont, California), Samet Yildirim, Raquel Orozco, and Min Zhu (former Boehringer Ingelheim) for their contribution and support of this project. The authors also acknowledge the Charles River Laboratories (Malvern, Pennsylvania) Viral Clearance Group for their assistance in viral material generation, execution of the experimental protocols, and sample titering.

CONFLICTS OF INTEREST

The authors declare no conflicts of interest.

DATA AVAILABILITY STATEMENT

The authors elect to not share data.

ORCID

Matthew Brown http://orcid.org/0000-0003-2556-2539

REFERENCES

ASTM. (2012). *E2888-12: standard practice for process for inactivation of rodent retrovirus by pH*. ASTM International. https://www.astm.org

Brorson, K., Krejci, S., Lee, K., Hamilton, E., Stein, K., & Xu, Y. (2003). Bracketed generic inactivation of rodent retroviruses by low pH treatment for monoclonal antibodies and recombinant proteins. *Biotechnology and Bioengineering*, *82*(3), 321–329. https://doi.org/10.1002/bit.10574

Brown, M. R., & Orozco, R. (2021). Utilizing bacteriophage to define the minimum residence time within a plug flow reactor. *Biotechnology Bioengineering*, 1–8. https://doi.org/10.1002/bit.27734

Brown, M. R., Orozco, R., & Coffman, J. (2020). Leveraging flow mechanics to determine critical process and scaling parameters in a continuous viral inactivation reactor. *Biotechnology and Bioengineering*, *117*(3), 637–645. https://doi.org/10.1002/bit.27223

Chinniah, S., Hinckley, P., & Connell-Crowley, L. (2016). Characterization of operating parameters for XMuLV inactivation by low pH treatment. *Biotechnology Progress*, *32*(1), 89–97. https://doi.org/10.1002/btpr.2183

Coolbaugh, M. J., Varner, C. T., Vetter, T. A., Davenport, E. K., Bouchard, B., Fiadeiro, M., Tugcu, N., Walther, J., Patil, R., & Brower, K. (2021). Pilot-scale demonstration of an end-to-end integrated and continuous biomanufacturing process. *Biotechnology and Bioengineering*. https://doi.org/10.1002/bit.27670

David, L., Bayer, M. P., Lobedann, M., & Schembecker, G. (2020). Simulation of continuous low pH viral inactivation inside a coiled flow inverter. *Biotechnology and Bioengineering*, *117*, 1048–1062. https://doi.org/10.1002/bit.27255

David, L., Maiser, B., Lobedann, M., Schwan, P., Lasse, M., Ruppach, H., & Schembecker, G. (2019). Virus study for continuous low pH viral inactivation inside a coiled flow inverter. *Biotechnology and Bioengineering*, *116*(4), 857–869. https://doi.org/10.1002/bit.26872

Gillespie, C., Holstein, M., Mullin, L., Cotoni, K., Caulmare, J., & Greenhalgh, P. (2019). Continuous in-line virus inactivation for next generation bioprocessing. *Biotechnology Journal*, *14*, e1700718. https://doi.org/10.1002/biot.201700718

Kateja, N., Nitika, N., Fadnis, R. S., & Rathore, A. S. (2021). A novel reactor configuration for continuous virus inactivation. *Biochemical Engineering Journal*, *167*, 107885. https://doi.org/10.1016/j.bej.2020.107885

Klutz, S., Lobedann, M., Bramsiepe, C., & Schembecker, G. (2016). Continuous viral inactivation at low pH value in antibody manufacturing. *Chemical Engineering and Processing: Process Intensification*, *102*, 88–101. https://doi.org/10.1016/j.cep.2016.01.002

Martins, D. L., Sencar, J., Hammerschmidt, N., Flicker, A., Kindermann, J., Kreil, T. R., & Jungbauer, A. (2020). Truly continuous low pH viral inactivation for biopharmaceutical process integration. *Biotechnology and Bioengineering*, *117*, 1406–1417. https://doi.org/10.1002/bit.27292

Orozco, R., Godfrey, S., Coffman, J., Amarikwa, L., Parker, S., Hernandez, L., & Fiadeiro, M. (2017). Design, construction, and optimization of a novel, modular, and scalable incubation chamber for continuous viral inactivation. *Biotechnology Progress*, *33*(4), 954–965. https://doi.org/10.1002/btpr.2442

Parker, S. A., Amarikwa, L., Vehar, K., Orozco, R., Godfrey, S., Coffman, J., Shamlou, P., & Bardliving, C. (2018). Design of a novel continuous flow reactor for low pH viral inactivation. *Biotechnology and Bioengineering*, *115*, 606–616. https://doi.org/10.1002/bit.26497

Schofield, M., & Johnson, D. (2018). Continuous low-pH virus inactivation: Challenges and practical solutions. *Genetic Engineering & Biotechnology News*, *38*, S13–S15.

How to cite this article: Brown, M., Godfrey, S., Creasy, A., Salm, J., & Fahrner, R. (2022). Continuous low pH viral inactivation: Operation and scaling strategy informs viral clearance study. *Biotechnology and Bioengineering*, 119, 2115–2121. https://doi.org/10.1002/bit.28117