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Approaches for Applying Perfusion Mimic in Ambr® 15 for Increased Understanding of Intensified Processes at the Microscale

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Introduction

Perfusion processes have gained popularity due to the possibility to reach higher cell densities and extend the protein production phase. Increasingly, these processes are adopted at the early stages of process development to ensure that clones, media and feeds taken forward are selected under the most representative conditions.

The Ambr® 15 Cell Culture system has been shown to provide improved results when compared to shake flask or shaking plate cultures from the high level of automation combined with reliable and independent process control for pH and DO. With low working volumes, the small scale stirred bioreactor vessels offer considerable advantages for screening of cell lines and media when running intensified processes, particularly in terms of costs.

To date, this method of processing has been a challenge at the microbioreactor scale, however new capabilities introduced in the Ambr® 15 Cell Culture Generation 2 system better support perfusion mimic applications. Two perfusion mimic protocols have been established and the results compared to show how different approaches can be applied to increase understanding of intensified processes at the microscale.

Methods

Two methods were evaluated; centrifugation and cell settling. Both follow traditional perfusion theory of exchanging spent media for fresh media at a semi-continuous rate, while retaining cells in the bioreactor. In both methods, sparged Ambr® 15 vessels were inoculated at a seeding density of 2.5e6 cells/mL and a 10 mL working volume. A perfusion rate of 1 vvd was used, and cell bleeding was performed to maintain the cell density at 20e6 cells/mL using the Rapid Vessel Drain feature of the Generation 2 liquid handler and a custom equation to determine the volume of culture to be removed. The process was run for approximately two weeks with samples for cell counts, metabolites, and titers taken daily. The Cedex HiRes was used for cell counts, and the Cedex Bio HT analyzer was used for titer quantification and metabolite analysis. A mAb-producing CHO DG44 cell line was grown in chemically defined, animal origin free (AOF) media, both developed by Sartorius Stedim Cellca. It is crucial to implement a constant flow of air as part of the gassing strategy to ensure that there is constant positive pressure into the vessels, thereby preventing backflow through the sparger into the clamp plate. Moreover, daily additions of antifoam are essential to preemptively prevent foam from accumulating on the surface of the culture.

Figure 1.

Process Parameter	Setpoint
Working volume	10 mL
DO setpoint	60%
pH setpoint	7.00 +/- 0.10
Stir speed	1200 rpm (increased to maintain DO setpoint if needed)
Gassing strategy	O_2 on demand up to 0.91 mL/min Constant air at 0.10 mL/min CO_2 on demand up to 1.24 mL/min
Foam control	Daily 20µL additions of 2% Antifoam C

Figure 2. Process parameters for both perfusion mimic methods evaluated.

Centrifugation Method

Centrifugation is one method of separating cells from the supernatant, whereby vessels are centrifuged to create a cell pellet, and the remaining supernatant is exchanged with fresh media at a whole vessel volume once a day (1 vvd). The steps in which the vessels are out of process control were performed in rapid succession to minimize the time of process disruption.

With the Ambr® 15 Cell Culture Generation 2 system, the supernatant was removed using the Rapid Vessel Drain, which allowed a quick (~5 seconds) aspiration of the entire supernatant from each vessel. Cell bleeding was performed using the Passage Culture Vessel step, which removed a volume of the culture from the vessel and replaced it with fresh media to reach a specified viable cell density (VCD).

For centrifugation, specially made 3D-printed adapters were used, each with the capacity to hold three Ambr® 15 vessels and together allowing up to 12 vessels to be centrifuged at a time. The angled design ensured that the cells pelleted in the corner of the vessel furthest away from the pH and DO spots, and also away from the sampling port.

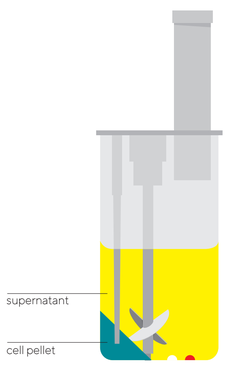

Figure 3. Centrifugation method in Ambr® 15 vessel.

This allowed for easy removal of the supernatant using the Rapid Vessel Drain function with minimal disturbance to the cell pellet. As the cell pellets grew larger due to the increased amount of cells over the course of the experiment, the relative centrifugal force (RCF) was also increased from 200 g up to 400 g to ensure that the cells were retained in the vessel.

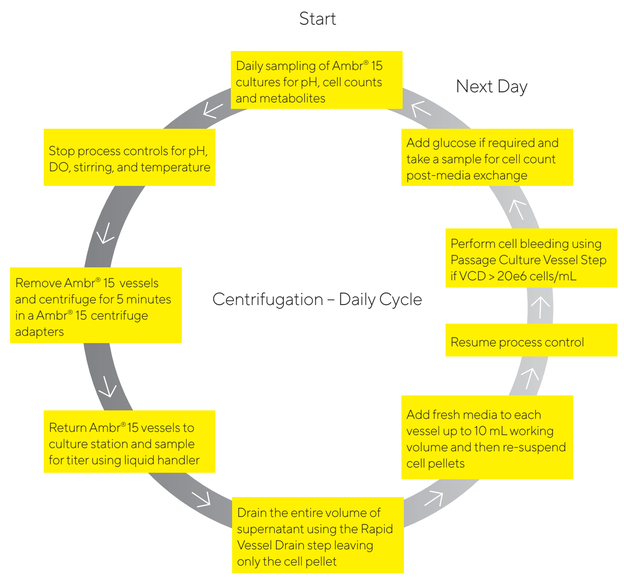

Figure 4. Daily cycle for steps performed in centrifugation method.

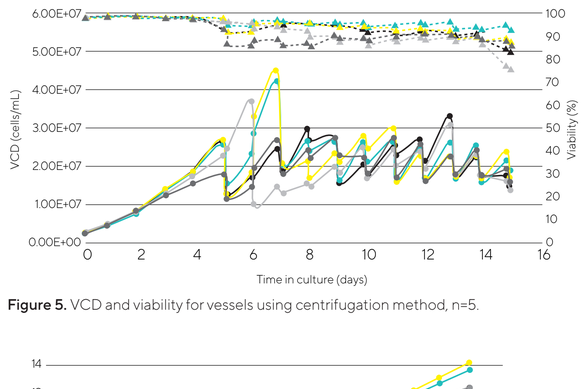

Figure 5. VCD and viability for vessels using centrifugation method, n=5.

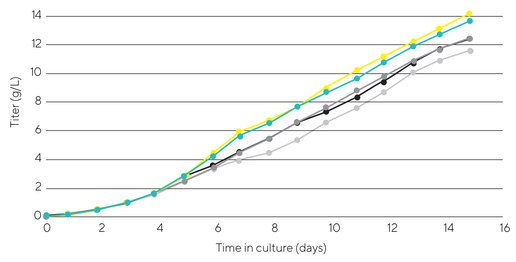

Figure 6. Cumulative titer for vessels using centrifugation method, n=5.

Cell Settling Method

For the cell settling method, stirring in the microbioreactor vessels was stopped to allow time for cells in the culture to settle to the bottom of the bioreactor before exchanging a portion of the supernatant with fresh media. A settling time of 35 minutes and a media exchange three times per day was found to provide the best conditions to minimize cell loss and maintain good cell viability. This meant exchanging one third of the working volume every 8 hours for a 1 vvd perfusion rate.

Samples for cell counts were taken once daily between the settling events, as was the cell bleeding. Cell bleeding was performed using the Passage Culture Vessel step, which exchanges a certain volume of culture with fresh media to target a specified VCD using the Rapid Vessel Drain. At each settling, titer samples were taken after 22 minutes, from an appropriate height to remove cell-free samples. After 35 minutes, the remaining top third of the culture volume was removed using the Rapid Vessel Drain, and fresh media was added back to each vessel to take the working volume back to 10 mL. The total settling time including media refill was 55 minutes for an entire culture station comprising 12 microbioreactors.

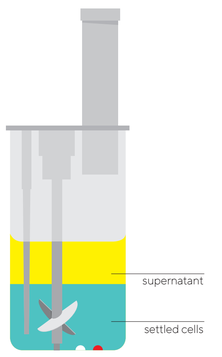

Figure 7. Cell settling method in Ambr® 15 vessel.

Steps for culture removal and media addition were written into the Ambr® 15 software and automated to be performed every 8 hours, which included overnight activities with no manual interactions or supervision required.

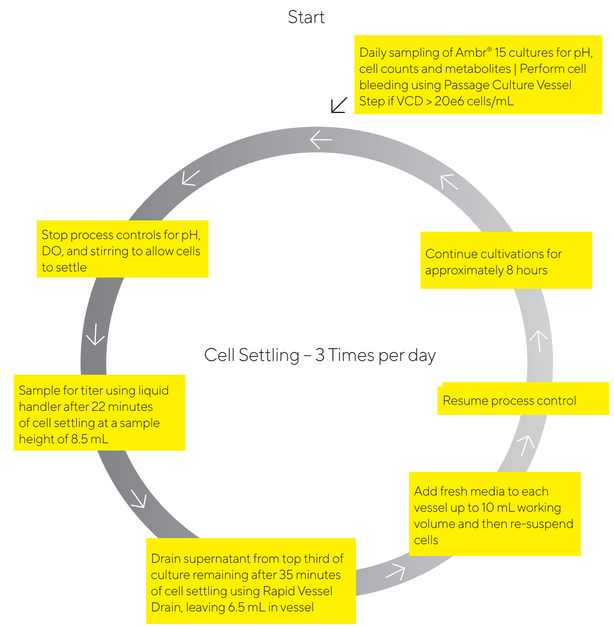

Figure 8. Daily cycle for steps performed in cell settling method.

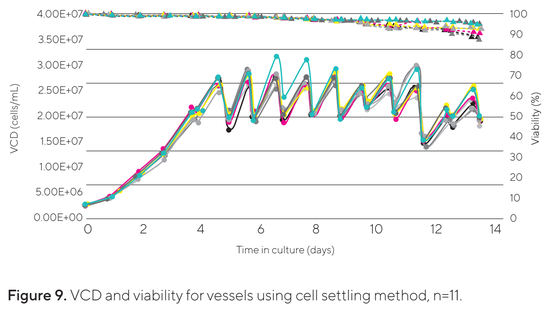

Figure 9. VCD and viability for vessels using cell settling method, n=11.

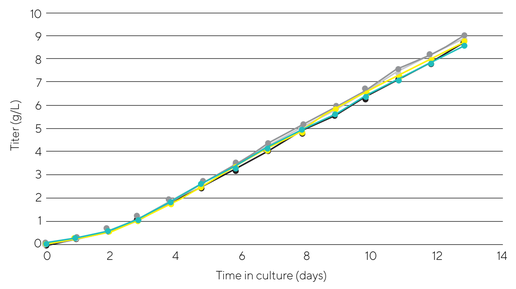

Figure 10. Cumulative titer for vessels using cell settling method, n=11.

High Cell Density DO Test

To demonstrate that the Ambr® 15 Cell Culture system is capable of supporting high cell densities typical of intensified processes, a test was performed to assess the feasibility of maintaining the DO setpoint. Microbioreactors were inoculated at two different high cell densities; 70e6 cells/mL and 90e6 cells/mL, and then cultivated with the same process setpoints. Cell count, viability, glucose concentration, and titer were analyzed to confirm a healthy culture. Media exchange was performed at a 1 vvd perfusion rate using the centrifugation method previously outlined, and glucose was added every 3 hours, based on daily consumption.

As the DO reading dropped from its setpoint due to the increased cell density, parameters to increase oxygenation in the vessel were adjusted step-wise until the DO was maintained. When this point was reached, the parameter would stay at this level for the remainder of the test. The levels were determined in the following sequence based on the potential negative impact on the cells as the level increases, shown in Figure 11.

Microbioreactor cultures inoculated at 70e6 cells/mL were able to maintain DO setpoint at level 2 (stir speed of 1600 rpm), while cultures inoculated at 90e6 cells/mL required a more rigorous change to level 4 (stir speed of 1800 rpm and ballast air decreased to 0.01 mL/min). The viable cell density, viability and titer for each of the microbioreactor cultures remained constant and results indicate that a change in parameter setting is capable of sustaining the increased oxygen demand of high cell densities.

Level	Parameter Change	Rationale
1	Increase stir speed to 1400 rpm	Increase kLa
2	Increase stir speed to 1600 rpm	Increase kLa
3	Decrease ballast air to 0.01 mL/min	Reduce competition of air with oxygen
4	Increase stir speed to 1800 rpm	Increase kLa
5	Change ballast gas to oxygen	Greater oxygen supply

Figure 11. Parameter changes implemented at each level and effect of each on the culture.

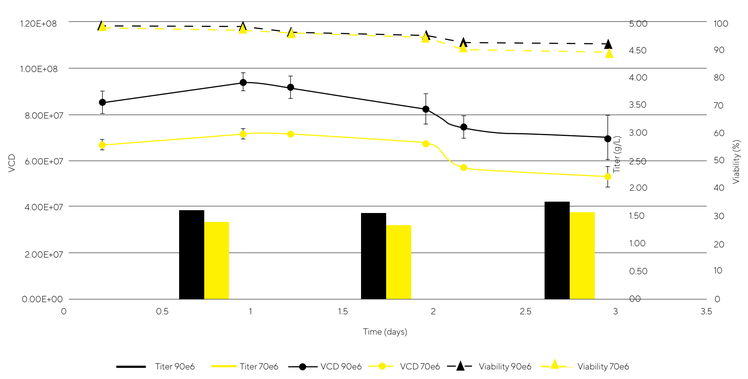

Figure 12. VCD, viability, and titer for vessels at 70e6 cells/mL and at 90e6 cells/mL using centrifugation method, n=3 for each condition.

Conclusion

Results from these experiments show that high throughput perfusion mimic processes can be achieved in the Ambr® 15 Cell Culture microbioreactors with good consistency amongst the replicates and with sustained cell viability and protein production.

The increased oxygen demand by the higher cell densities can be met, while tests indicate that even higher cell densities may be supported.

In addition, Ambr® 15 Cell Culture Generation 2 system provides key features supporting perfusion mimic protocols and allows for more efficient and automated approaches to be applied when undertaking intensified process studies.

The two methods presented provide different options for carrying out perfusion mimic studies depending on the feasibility of each in individual laboratories.

Optimization of perfusion mimic protocols together with comparison of data generated across different scales would further support implementation of these methods for scale-down screening of clones under intensified conditions.