IMPACT OF BIOREACTOR CONTROL PARAMETERS ON AAV PRODUCTION

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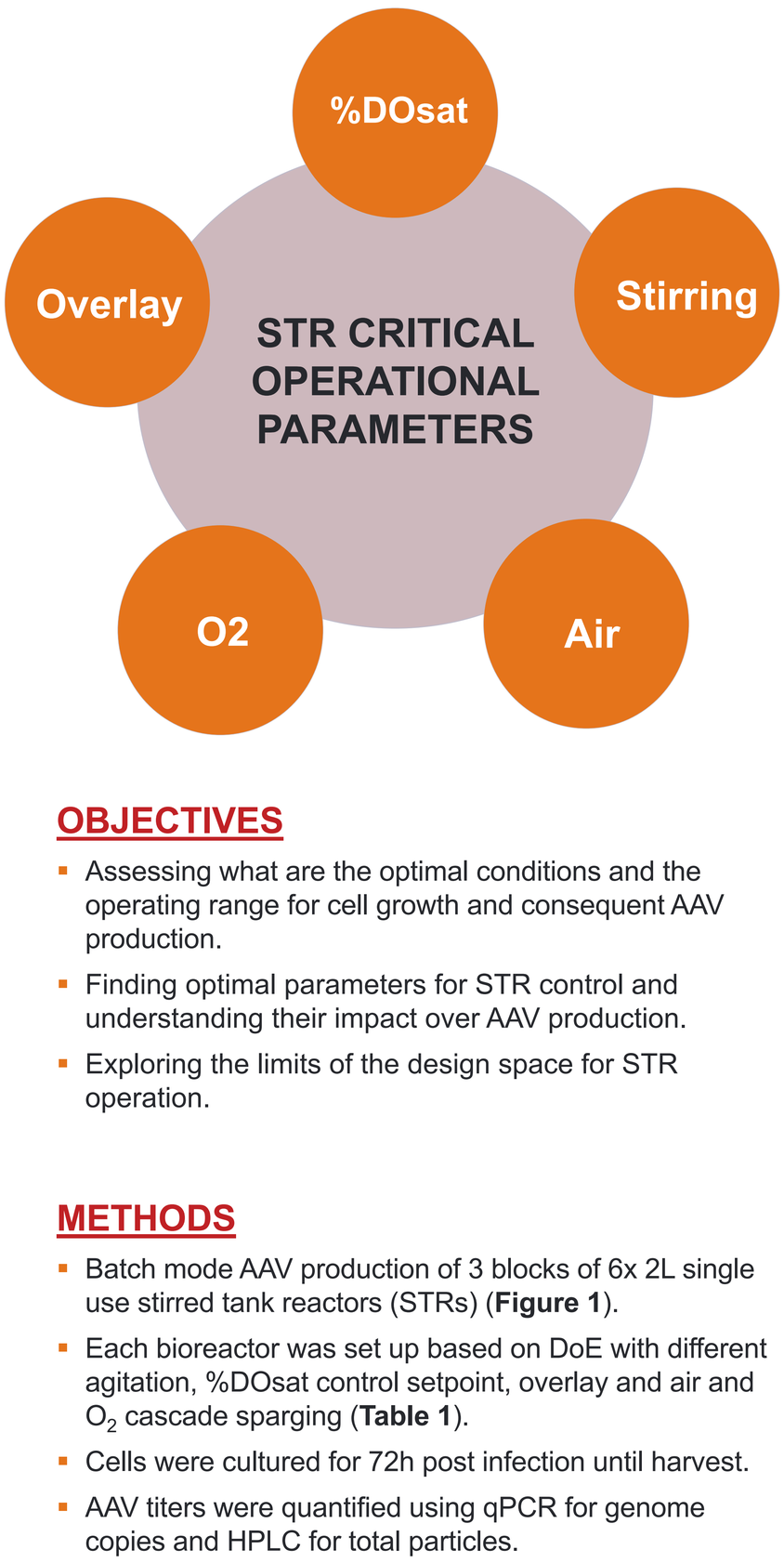
BACKGROUND

Optimal cell culture environment is critical for improving recombinant AAV production; arguably the most important factor to control in the bioreactor is oxygen supply. The effect of bioreactor parameters over baculovirus infected cells was assessed in DoE format in bench scale single-use stirred tank reactors. Parameters such as cascade type, stirring speed, gassing and dissolved oxygen setpoint were tested. The best conditions were identified to achieve the optimal AAV production process.



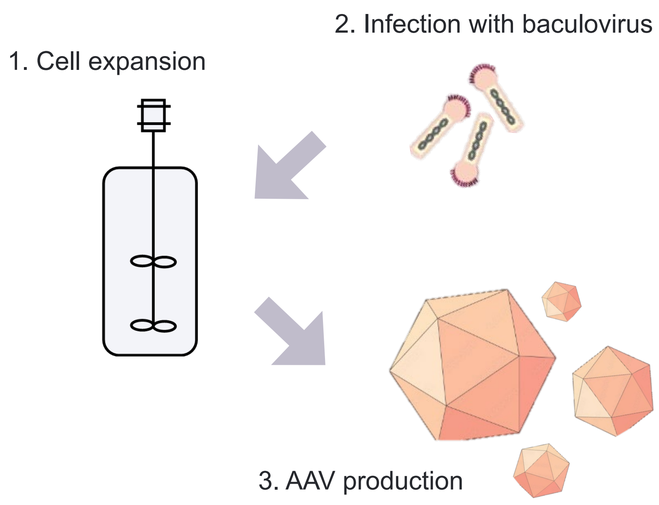
OBJECTIVES

- Assessing what are the optimal conditions and the operating range for cell growth and consequent AAV production.
- Finding optimal parameters for STR control and understanding their impact over AAV production.
- Exploring the limits of the design space for STR operation.

METHODS

- Batch mode AAV production of 3 blocks of 6x 2L single use stirred tank reactors (STRs) (**Figure 1**).
- Each bioreactor was set up based on DoE with different agitation, %DOsat control setpoint, overlay and air and O_2 cascade sparging (**Table 1**).
- Cells were cultured for 72h post infection until harvest.
- AAV titers were quantified using qPCR for genome copies and HPLC for total particles.

Figure 1. AAV production using BEVS (Baculovirus Expression System)



STR	Block	Agitation	Airflow	%DOsat	Overlay	Cascade
1	1	low	low	high	low	I
2	1	high	low	low	medium	I
3	1	medium	low	low	low	II
4	1	high	high	low	high	II
5	1	low	high	high	medium	II
6	1	medium	high	high	high	I
7	2	low	low	high	high	II
8	2	high	medium	high	high	II
9	2	high	high	low	low	I
10	2	high	high	high	low	I
11	2	low	low	low	high	II
12	2	low	medium	low	low	I
13	3	medium	medium	medium	medium	II
14	3	low	high	medium	low	II
15	3	high	low	medium	high	I
16	3	high	low	high	low	II
17	3	medium	medium	medium	medium	I
18	3	low	high	low	high	I

Table 1. DoE design

- Cascade I: Gas replacement strategy. Oxygen substitutes air in the sparging mixture.
- Cascade II: O_2 addition strategy. Pure oxygen added on top of air ballast.

RESULTS

Cell expansion

- %DOsat setpoint, cascade setting (O_2 and air), stirring and overlay do not have a significant impact on achieving target VCD.
- High airflow sparging has a major negative effect on cell growth **(Figure 4)**, which can be related to hydrodynamic stress and pCO_2 (not shown) stripping.
- Block effect observed, future work run in parallel, for example AMBR250.

Figure 2. VCD and viability at time of infection

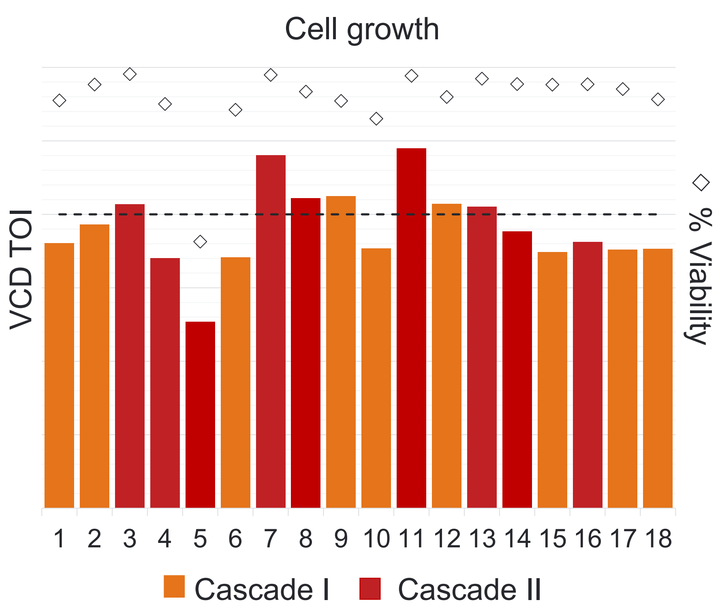
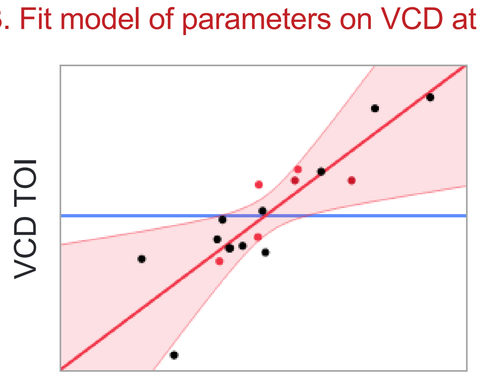


Figure 3. Fit model of parameters on VCD at TOI



Predicted RMSE=0.1707 RSq=0.78 PValue=0.0120

Figure 4. Effect summary for cell growth

Source	LogWorth	PValue
Block	2.300	0.00501
Airflow	1.811	0.01546
DO	1.185	0.06533
Cascade	0.613	0.24354
Stirring	0.228	0.59163
Overlay	0.142	0.72064

AAV production

- The most affecting parameter on AAV titer was the selected cascade, similarly to cell growth experiments cascade could be a major source of hydrodynamic stress and pCO_2 stripping (**Figure 5**).
  - STRs run with cascade I yielded about 50% less genome copies/mL comparing to our usual yield.
- Negative impact of stirring above standardly used speed was observed (**Figure 7**).
- Overlay, airflow and %DOsat setpoint did not play a major role in STR control over AAV production within explored ranges.

Figure 5. AAV production

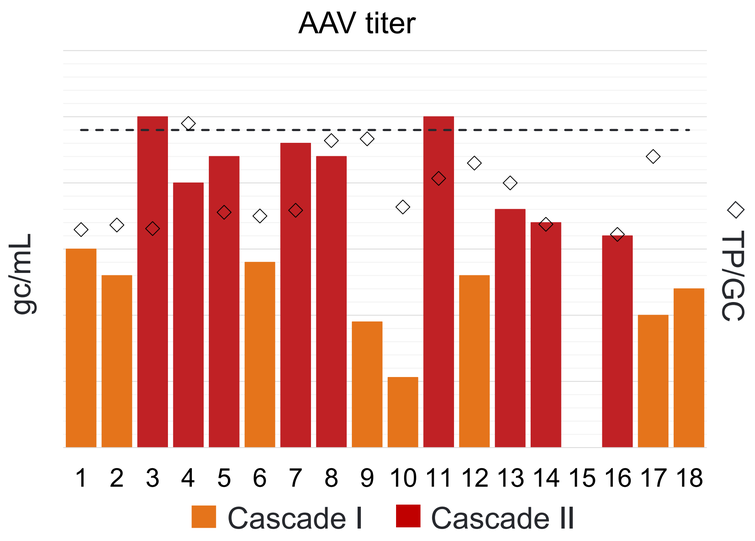
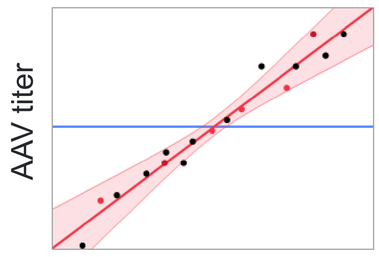


Figure 6. Fit model of STR controlling parameters on AAV titer



Predicted RMSE=0.1475 RSq=0.96 PValue=<.0001

Figure 7. Effect summary for AAV production

Source	LogWorth	PValue
Cascade	5.229	0.00001
Stirring	2.143	0.00720
Block	2.135	0.00733
Overlay	1.400	0.03984
Airflow	0.836	0.14588
DO	0.428	0.37288
VCD TOI	0.227	0.59343

CONCLUSION

- Significant differences in cell growth were observed under various STR control parameters.
- Direct sparging of air contributed the most of the studied parameters to the cell growth limitations, potentially due to hydrodynamic stress and pCO_2 stripping.
- *ExpressSF+* growth was not affected by different %DOsat, overlay or agitation within explored ranges.

→ Insect cells are more resistant to stress from agitation than to stress from bursting bubbles caused by aeration

- AAV production was not influenced by %DOsat set-point, agitation speed, overlay or airflow within explored range.
- Cascade settings of air and O_2 had a major effect on AAV production.

REFERENCES

1. Strobl, Florian, et al. "High shear resistance of insect cells: the basis for substantial improvements in cell culture process design." *Scientific Reports* 11.1 (2021): 1-11.
2. Palomares, Laura A., and Octavio T. Ramirez. "The effect of dissolved oxygen tension and the utility of oxygen uptake rate in insect cell culture." *Cytotechnology* 22.1 (1996): 225-237.

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