



# BSUITE 1: Analysis of Perfusion on CHO Cells

# Overview

- Introduction
  - Bioprocessing
- Upstream
  - Perfusion
  - Analysis
- Downstream
  - Bradford Assay
- Future Development

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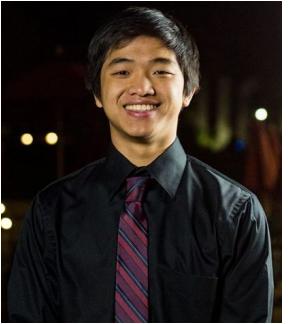


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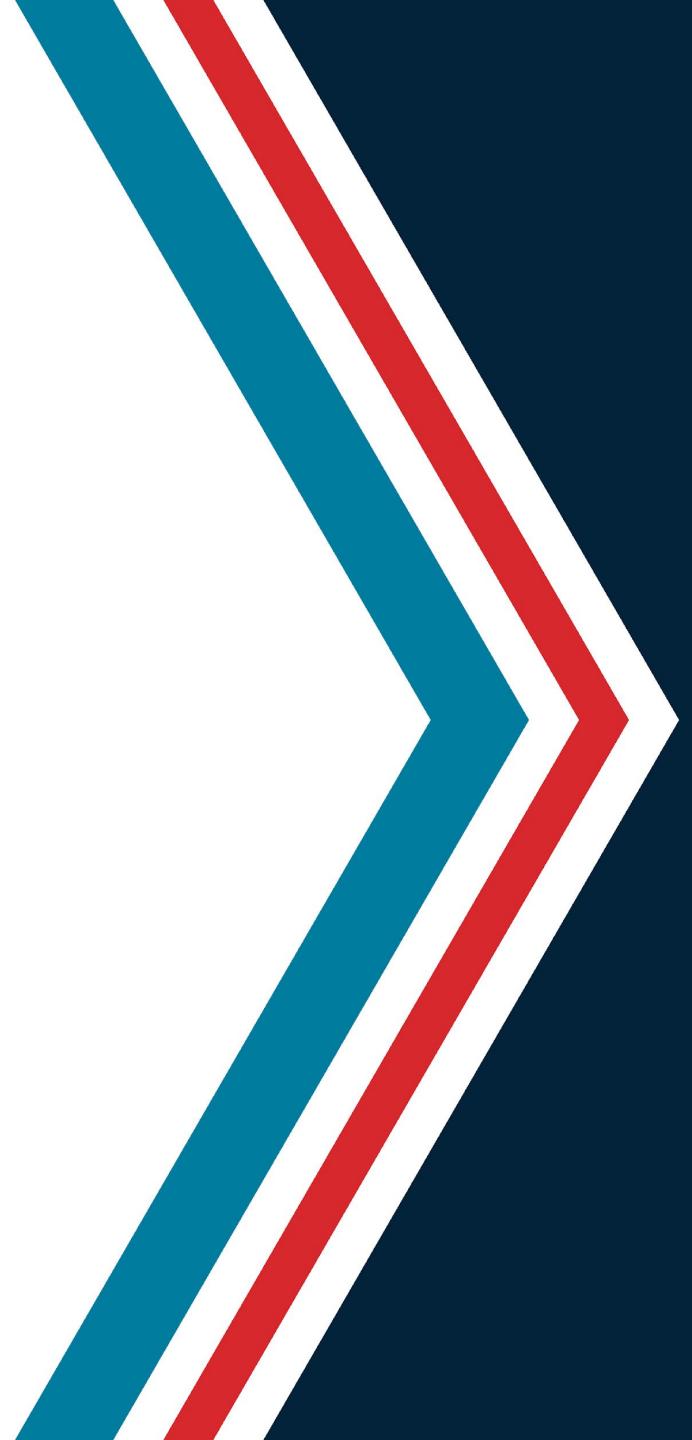
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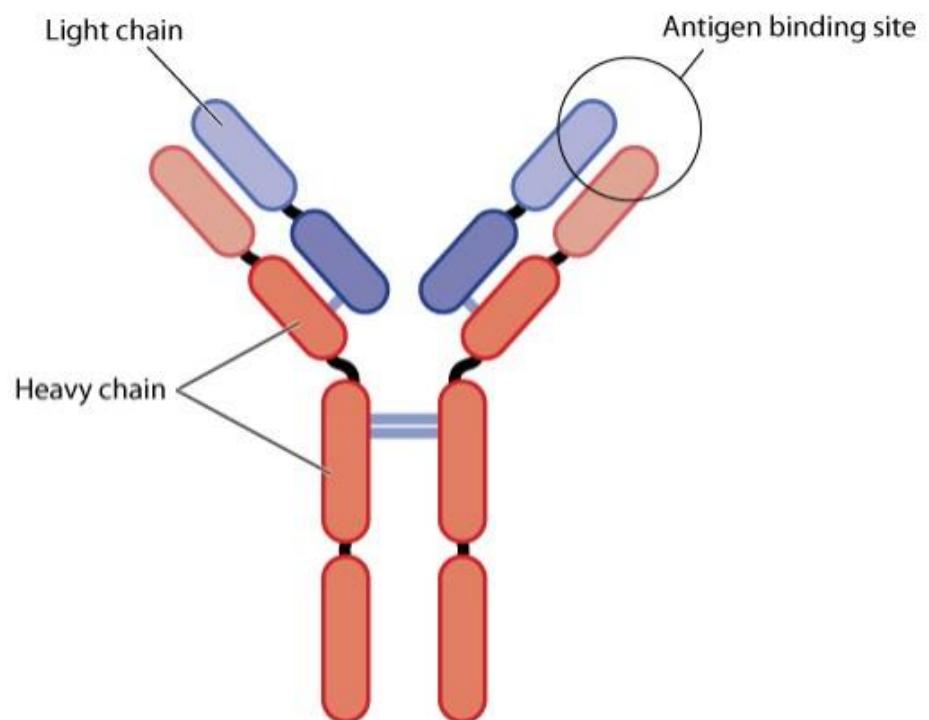


# Introduction to Bioprocessing



# Bioprocessing

- Bioprocessing: using living cells or components to obtain a desired product
- Monoclonal Antibodies (mAbs)



# Cell Lines

- Cell Lines
  - Yeast
  - Bacterial
  - Chinese Hamster Ovary cells
  - CHO - S cells

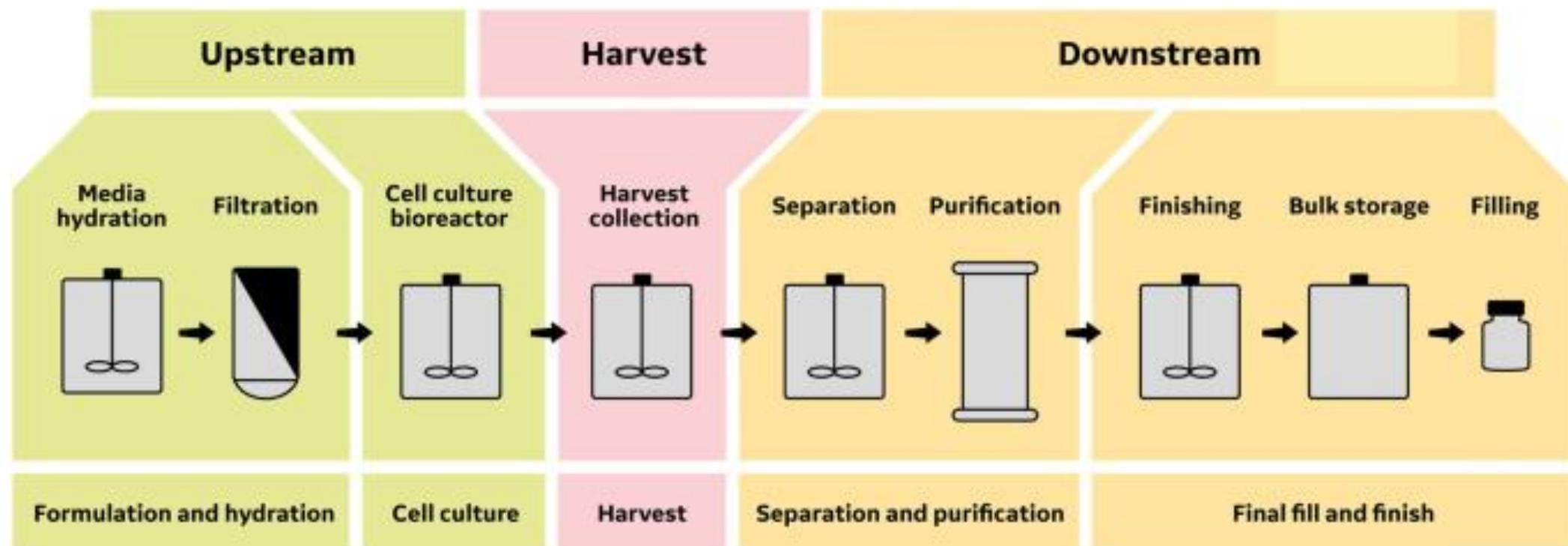


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# KGI Overview of Upstream & Downstream Bioprocessing

Bioprocess flow diagram, simplified



# Modes of Operation



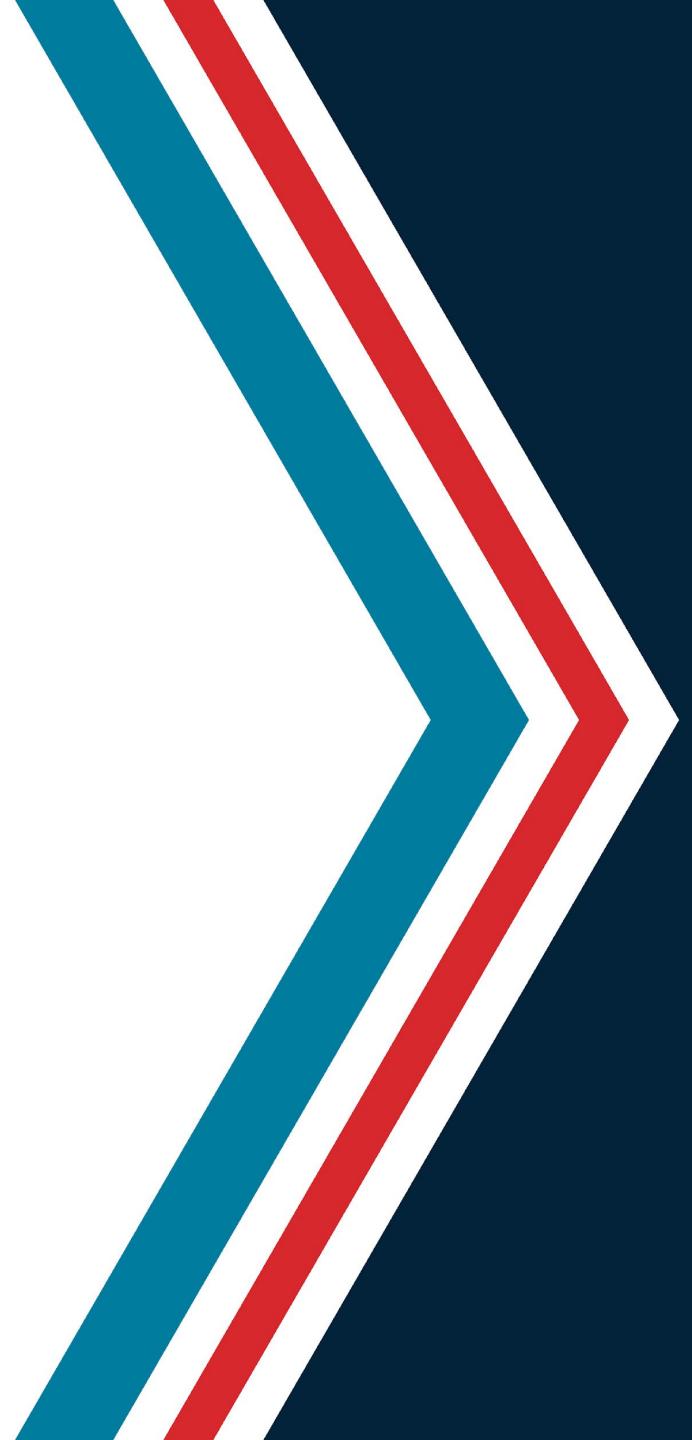
# CHO Cell Line

- According to Tapia et al. 2016, higher cell concentration yields higher virus production

Process Type	Peak cell conc. [10^6 cells/mL]	Specific growth rate [per day]	Specific productivity	Yield [g/L]	Reference
Batch	1.3	0.8	>2	0.04	Lee et al. (2005)
	8.5	0.7	>50	0.9	Reinhart et al. (2015)
Fed-batch	>10	0.7	57	>1	Zboray et al. (2015)
	23.9	0.7	>50	>5.8	Reinhart et al. (2015)
	25;26	n.a.	35; 49	10; 13	Huang et al. (2010)
Perfusion	27-33.5	n.a.	>2	0.09-0.015	Lee et al. (2005)



# Upstream



# Perfusion

Operation Modes	Advantage	Disadvantage
<b>Perfusion</b>	<ul style="list-style-type: none"><li>• Higher cell density</li><li>• Cell culture maintenance</li><li>• Highly suitable to unstable product</li></ul>	<ul style="list-style-type: none"><li>• Unreliable equipment<ul style="list-style-type: none"><li>• Scale up</li><li>• Cost</li><li>• Contamination</li></ul></li></ul>
<b>Fed batch</b>	<ul style="list-style-type: none"><li>• Produces higher yields</li><li>• Efficient process characterization</li><li>• reliable</li></ul>	<ul style="list-style-type: none"><li>• Waste products</li><li>• contamination</li><li>• periodically input of media</li></ul>

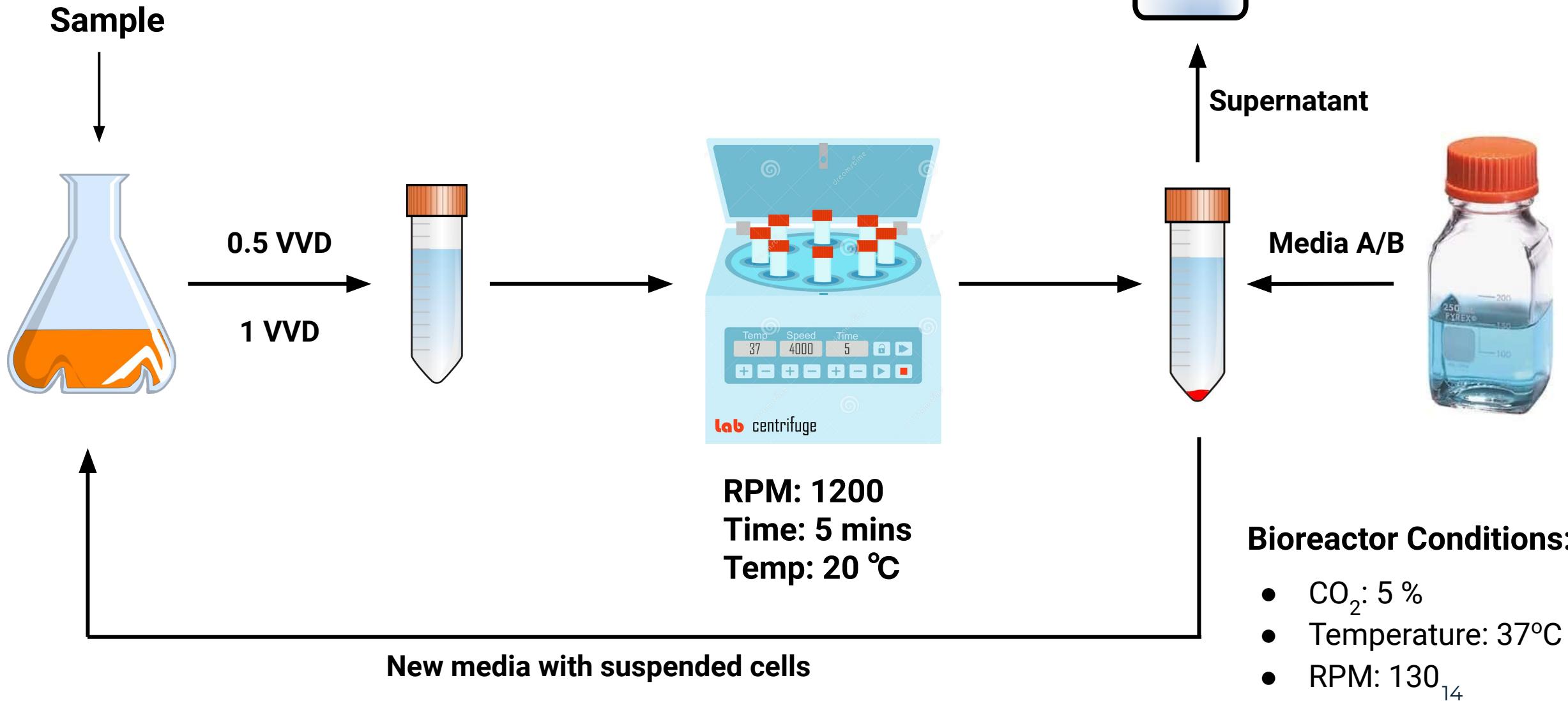
# Goals for Use of Perfusion for Upstream Processing

- Obtain higher cell density - greater than  $5 \times 10^6$  cells/mL compared to  $2 \times 10^6$  in fed-batch cultures
- Get reliable results with low infection rates
- Understand the results of the feed media A and media B to CHOS cells



<https://www.quimigen.com/en/flasks-for-mammalian-insect-cells-3765/optimum-growth-2-8l-flask-sterile-269000113.html>

# Method



# Perfusion Conditions

Condition	Media	Day 0-2 (Inoculation)	Day 2-3 (Perfusion starts)	Day 4-6	Day 7-8
1	A (PROCHO + Anti-clump)	<b>Flask Working Volume 35 mL</b>  <b>Cell Concentration <math>0.5 \times 10^6</math> cells/mL</b>	0.5 VVD	1 VVD	1 VVD
2			0.5 VVD	1 VVD	1 VVD
3			0.5 VVD	1 VVD	1 VVD + feed
4			1 VVD		
5			Control		
6			1 VVD		
7	B (without anti-clump)		Control		

# Materials



PPE

Shaker flasks  
(various sizes)Falcon 50 mL  
Centrifuge Tubes

Pipette gun

Sterile  
pipettes500 mL  
beakerMedia A/  
BBleach,  
EtOHLaminar Flow  
Hood37 °C  
water bathBeckman Coulter  
AllegraTM 64R  
CentrifugeReach-in CO2  
incubator

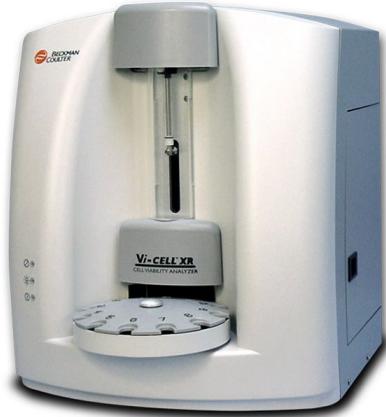
# Bioprofiling

**Nova**



pH      Ammonia  
Lac      Glu

**ViCell**



VCD  
% viability

**Osmometer**



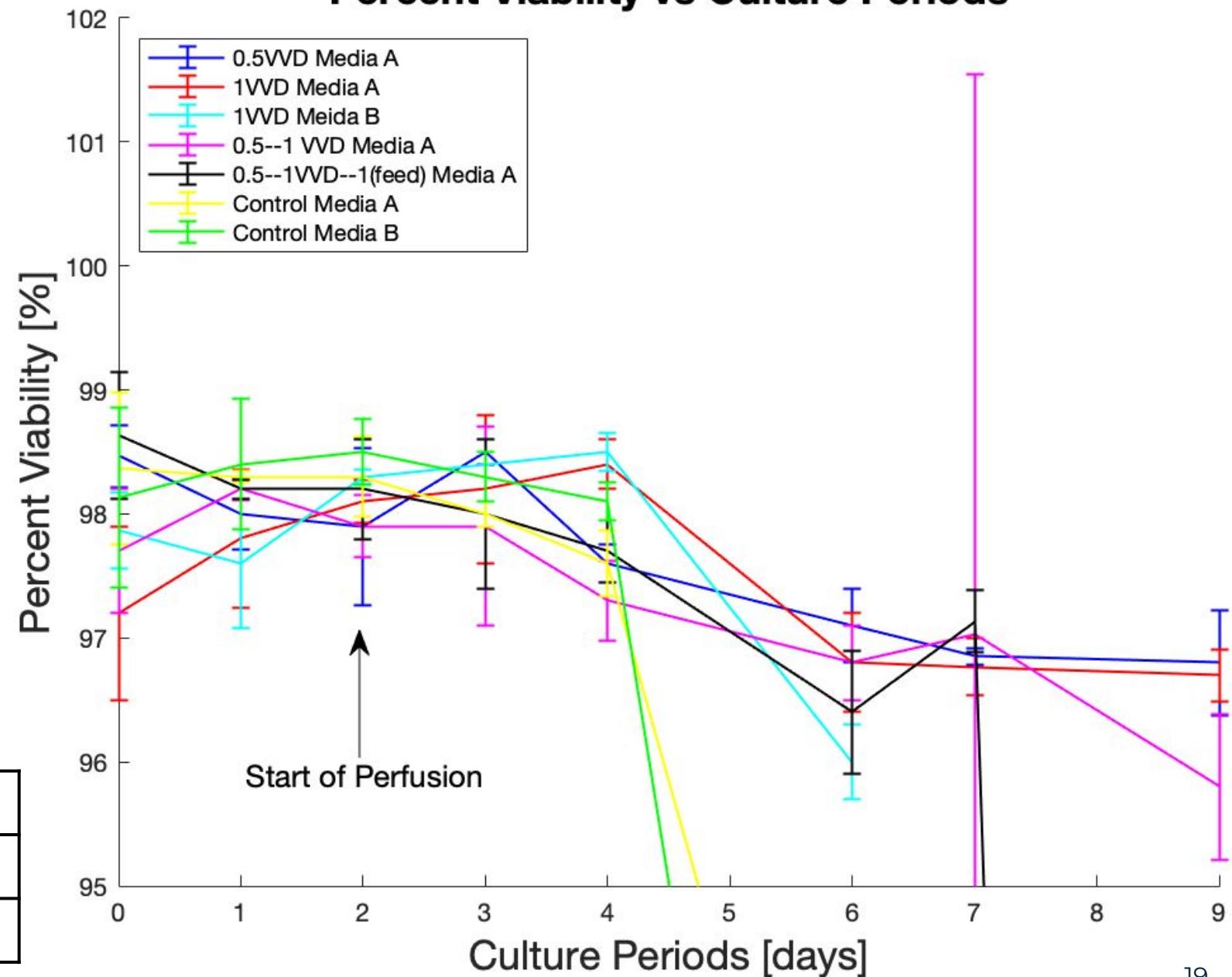
Osmolality

# Results and Data Analysis

## Approach

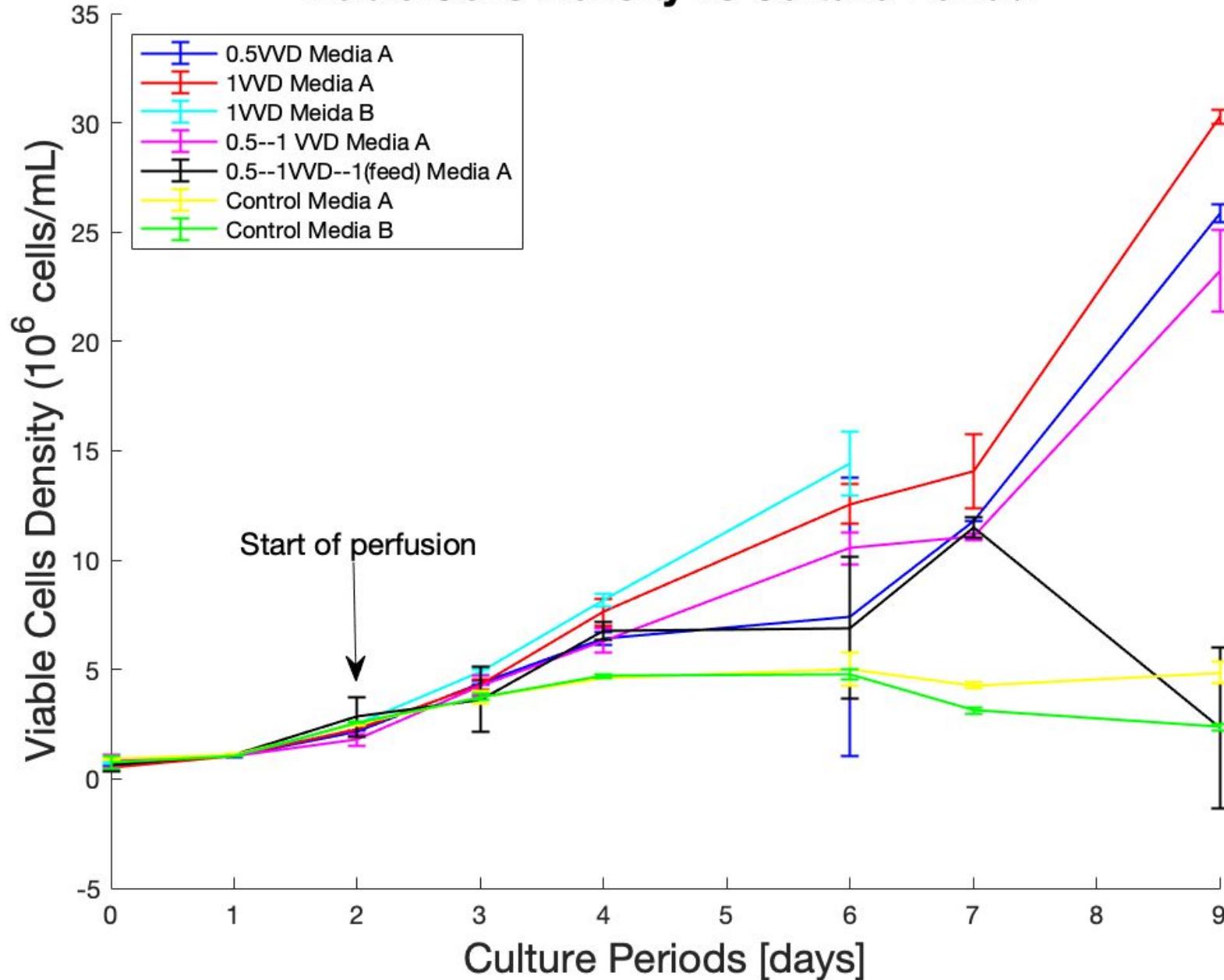
- **Graph the important parameters versus culture periods (days)**
  - Osmolality, pH (see in Appendix)
  - Glutamine, Lactate, Ammonia
  - VCD, % viability, iVCD
  - Doubling time, Specific Growth Rate
- **Seven conditions, three shaker flasks each**
  - Calculate the average value and the standard deviation
  - Construct graphs with error bars, one standard deviation (1SD) on each side
- **Graphing tool: MATLAB**

## Percent Viability vs Culture Periods



Day 9	Percent Viability [%]
Control Media A	70.9
Control Media B	41.6

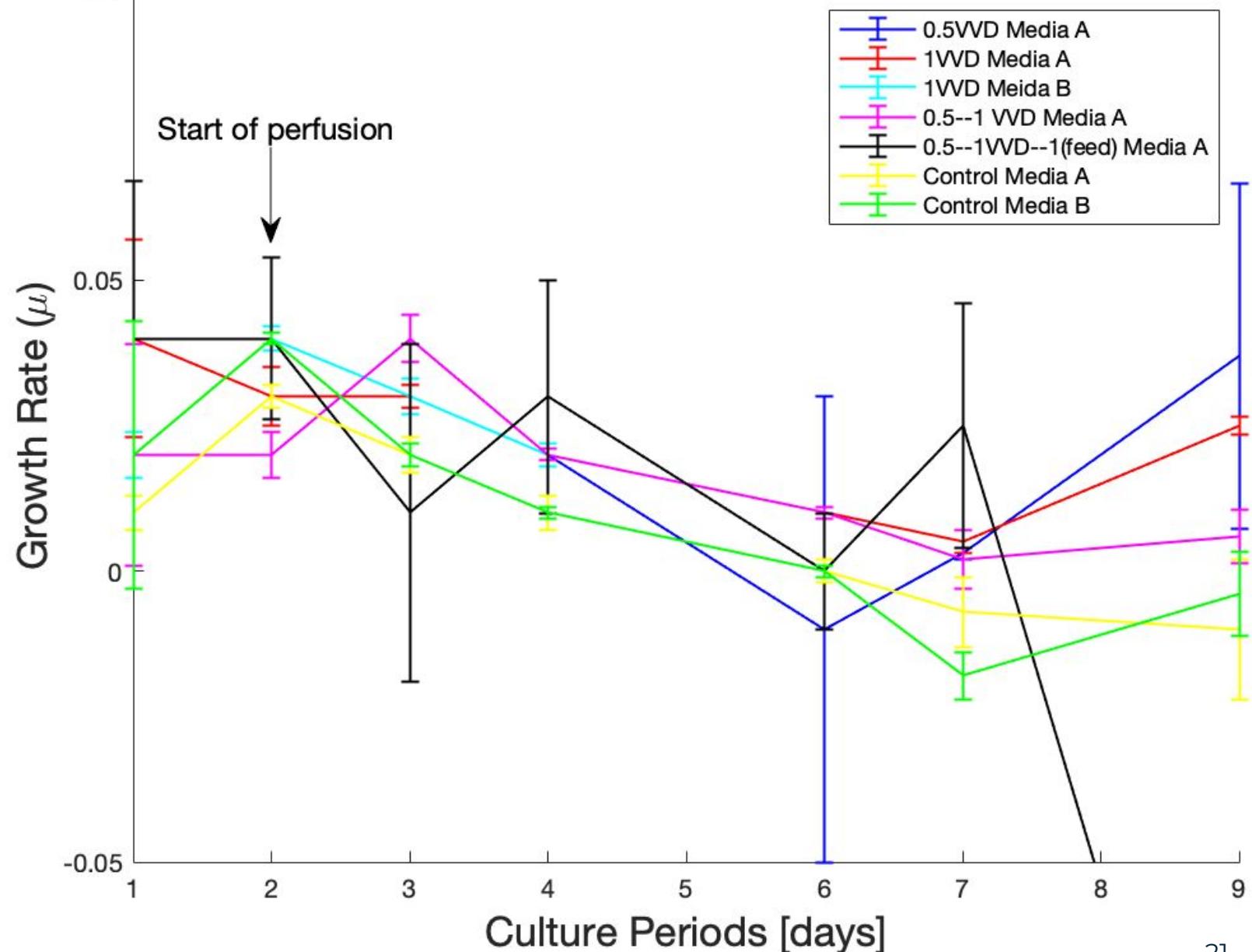
## Viable Cells Density vs Culture Period



Literature value of CHO cells growth rate: 0.0346

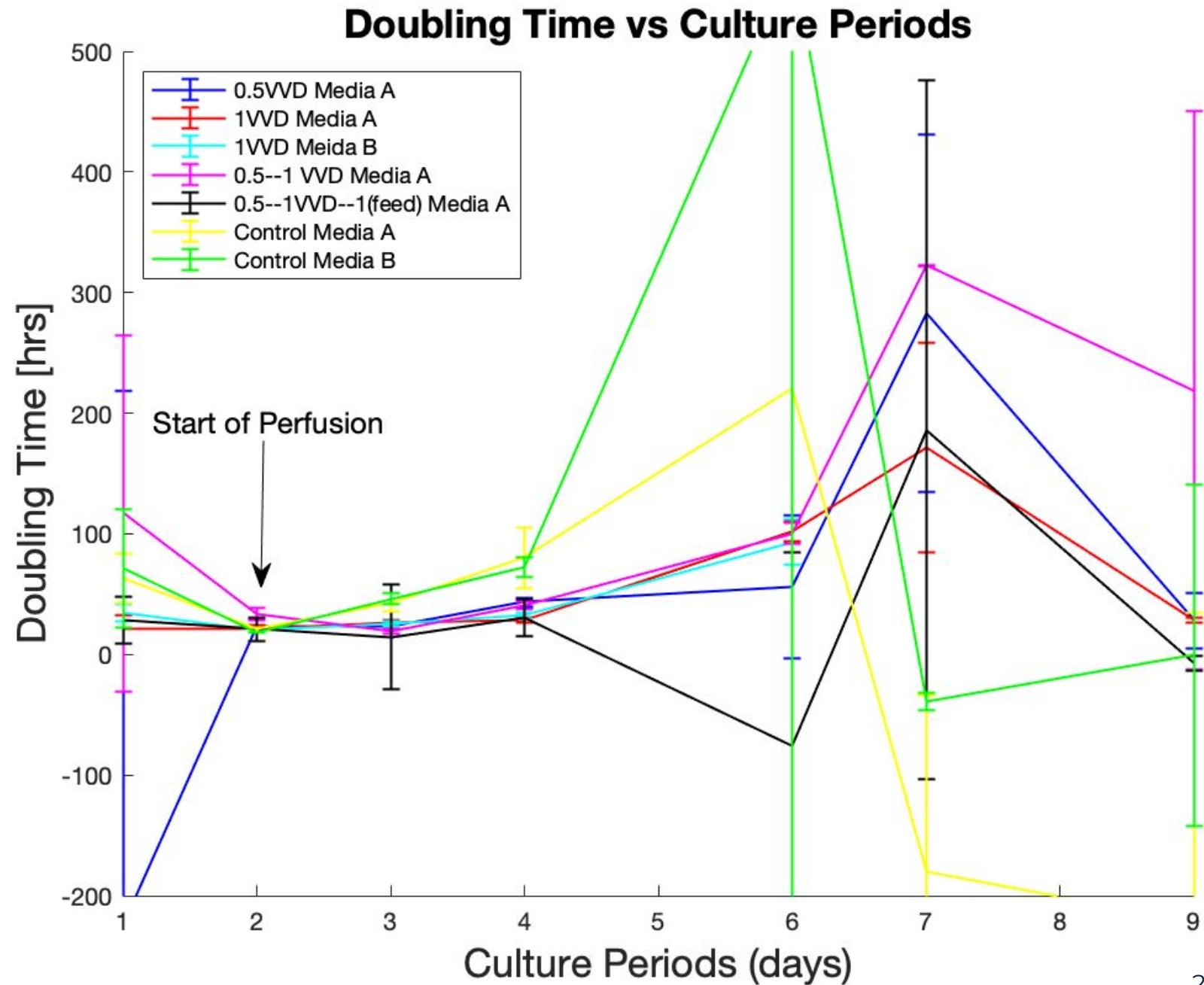
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## Growth Rate ( $\mu$ ) vs Culture Periods

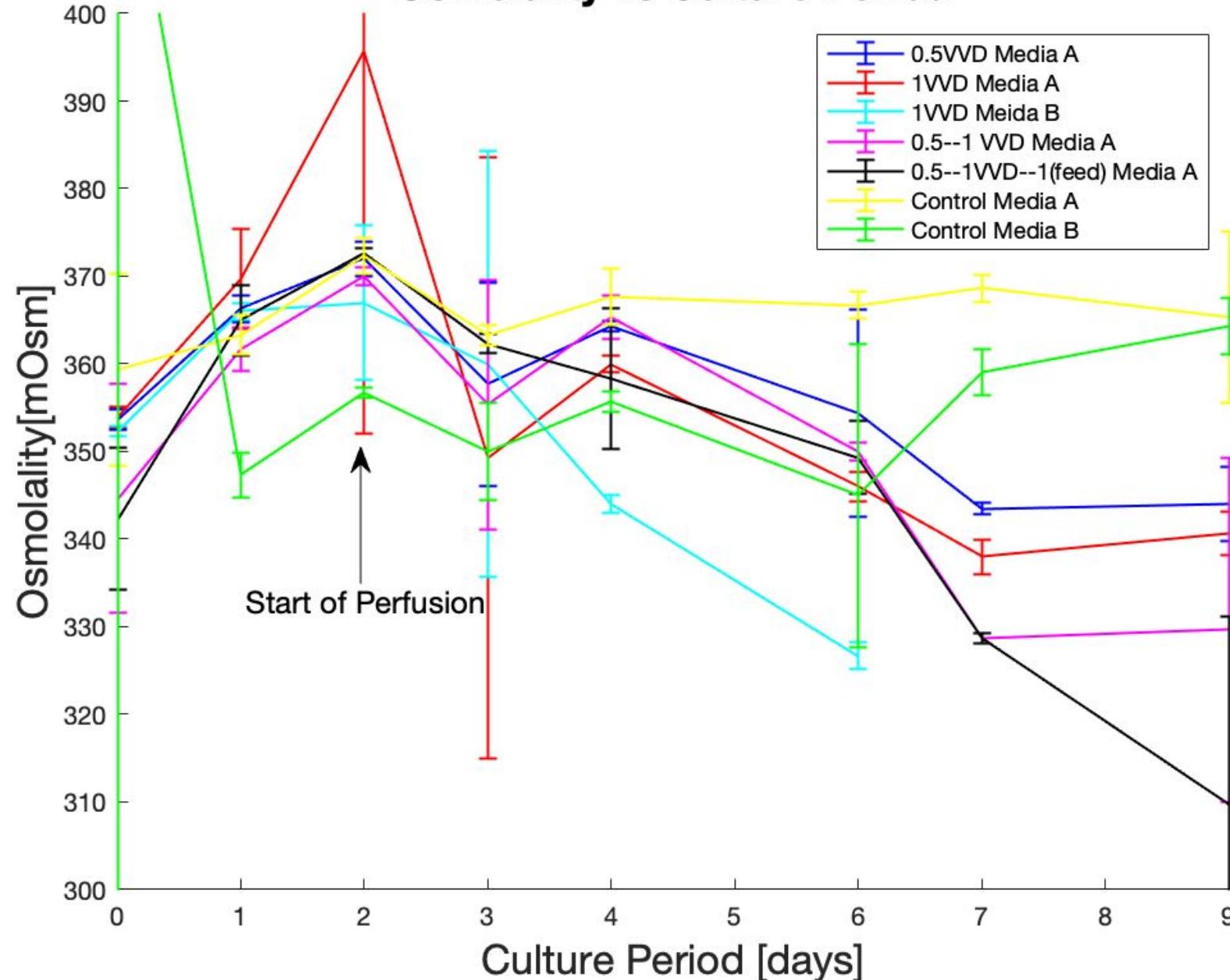


Literature value of CHO cells doubling time: 20 hrs

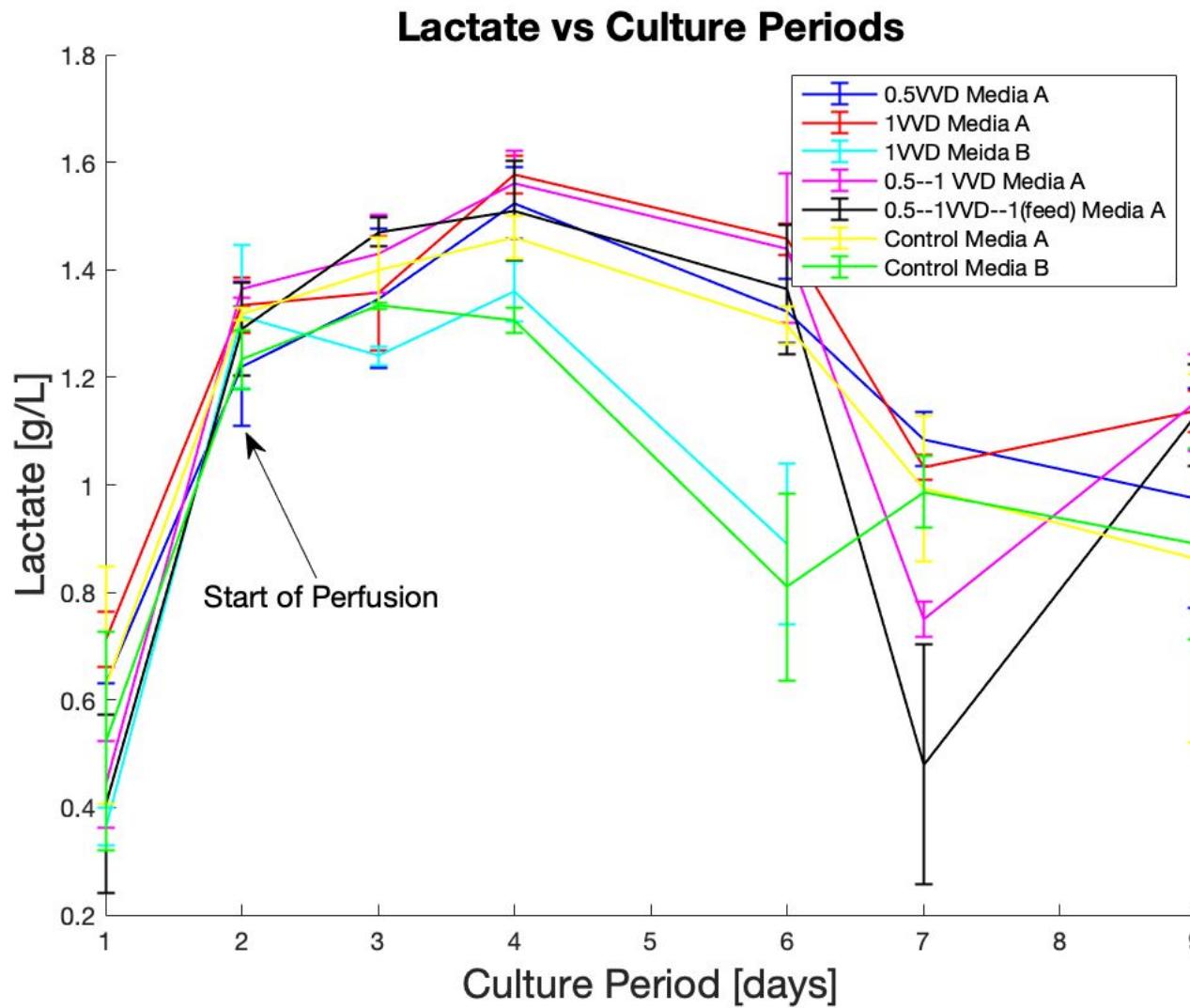
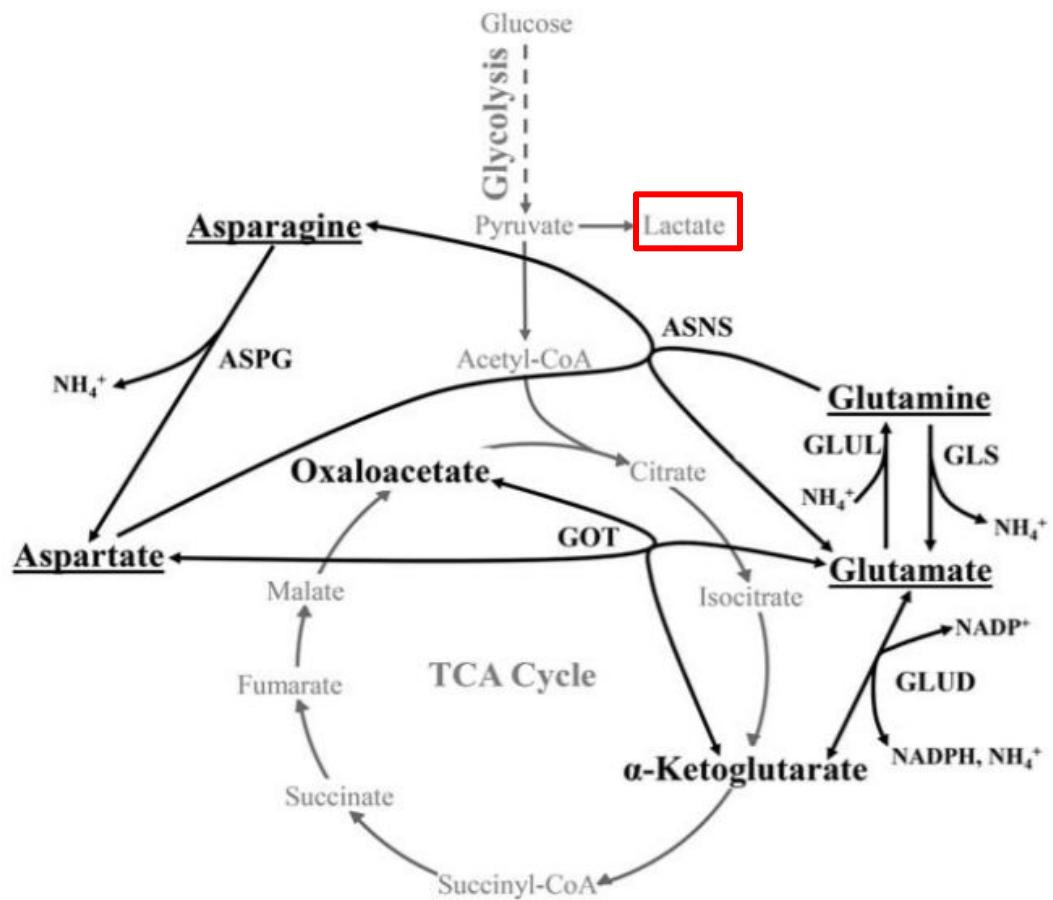
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## Osmolality vs Culture Period

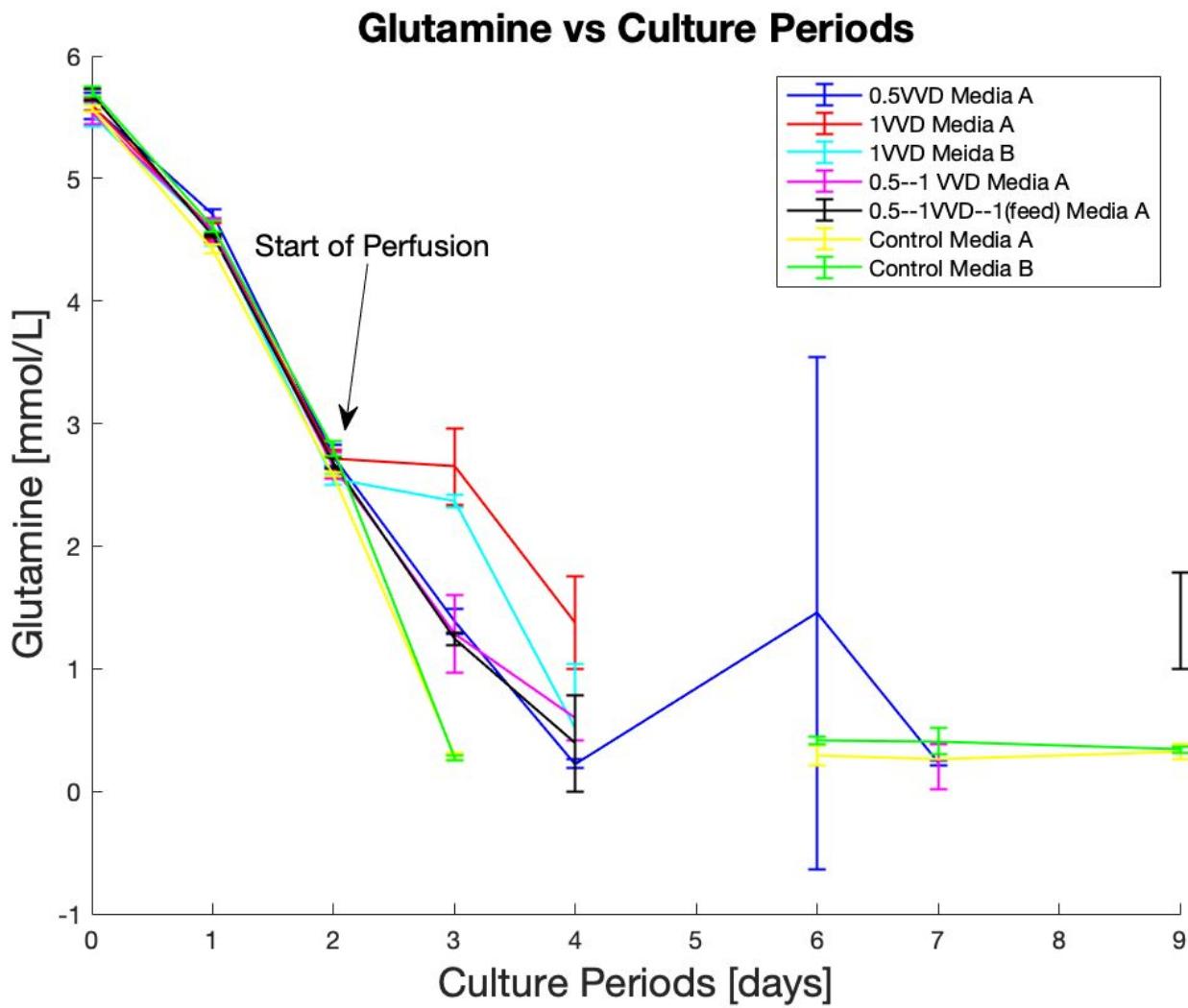
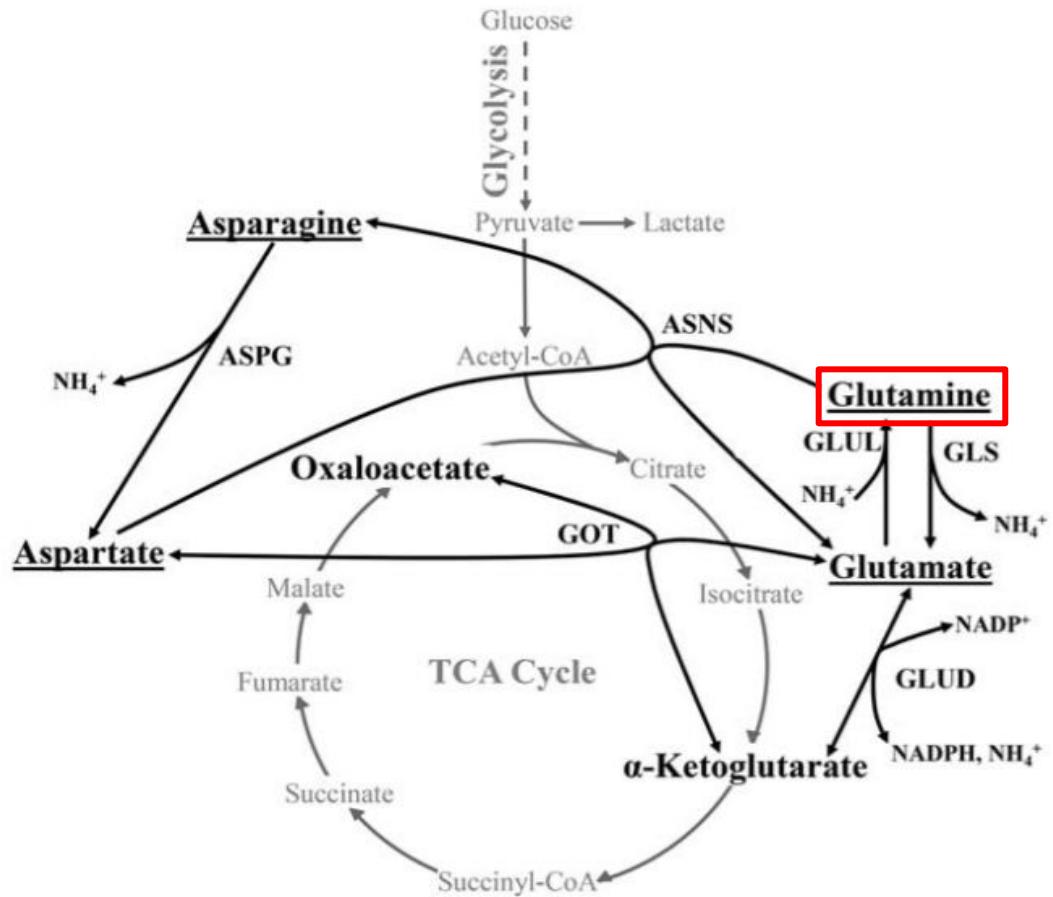


# Metabolic Pathway<sup>[1]</sup>



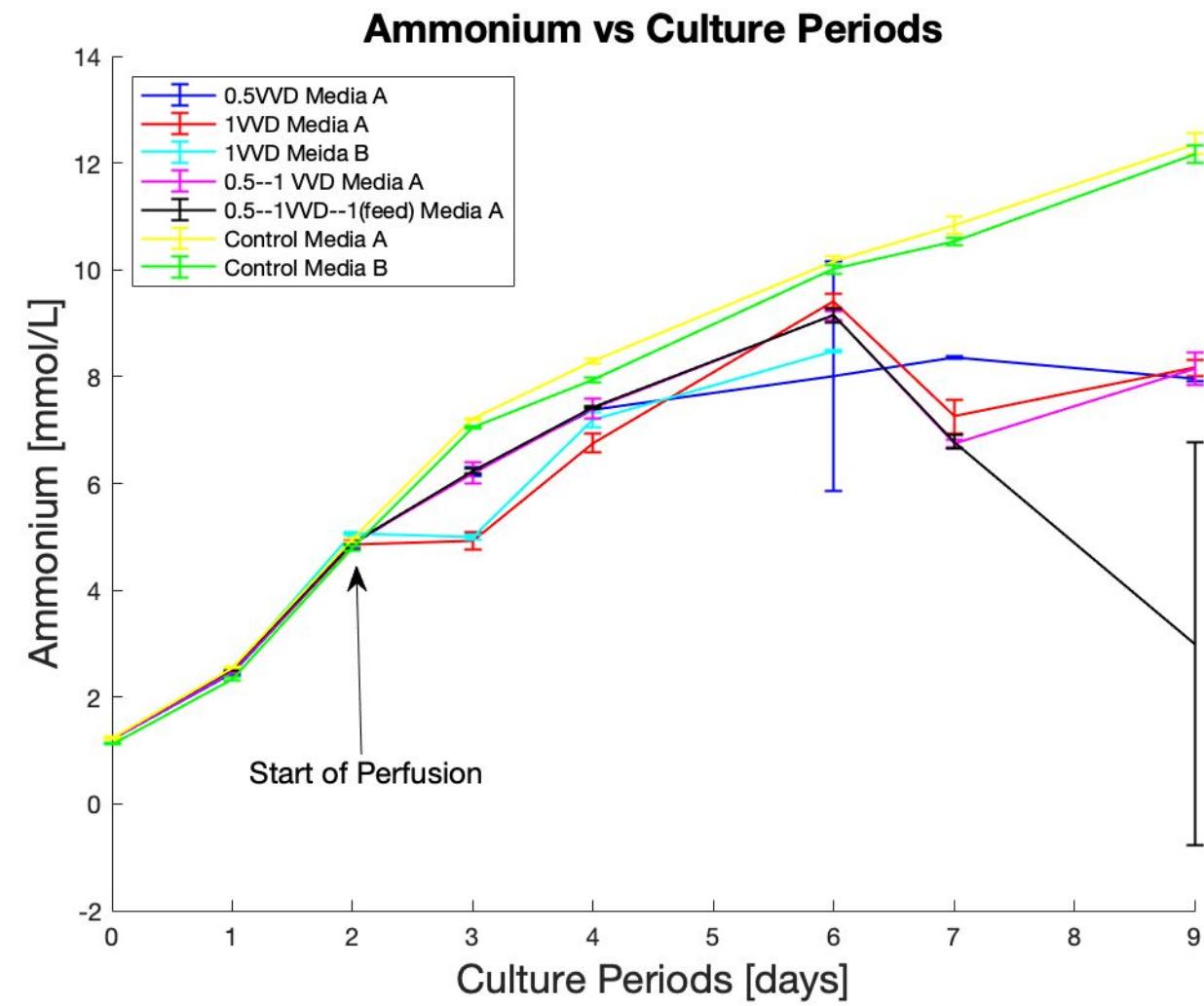
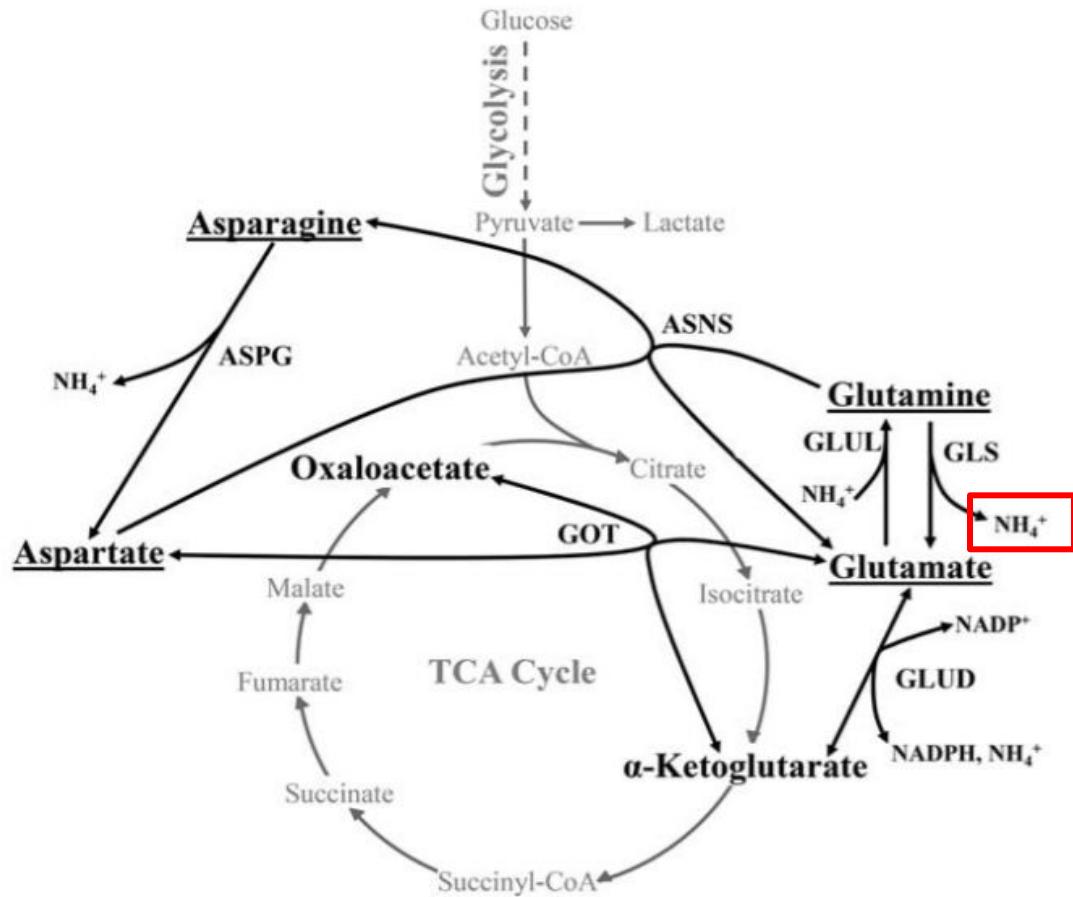
[1] Lin, H., Leighty, R. W., Godfrey, S., & Wang, S. B. (2017). Principles and approach to developing mammalian cell culture media for high cell density perfusion process leveraging established fed-batch media. *Biotechnology progress*, 33(4), 891-901.

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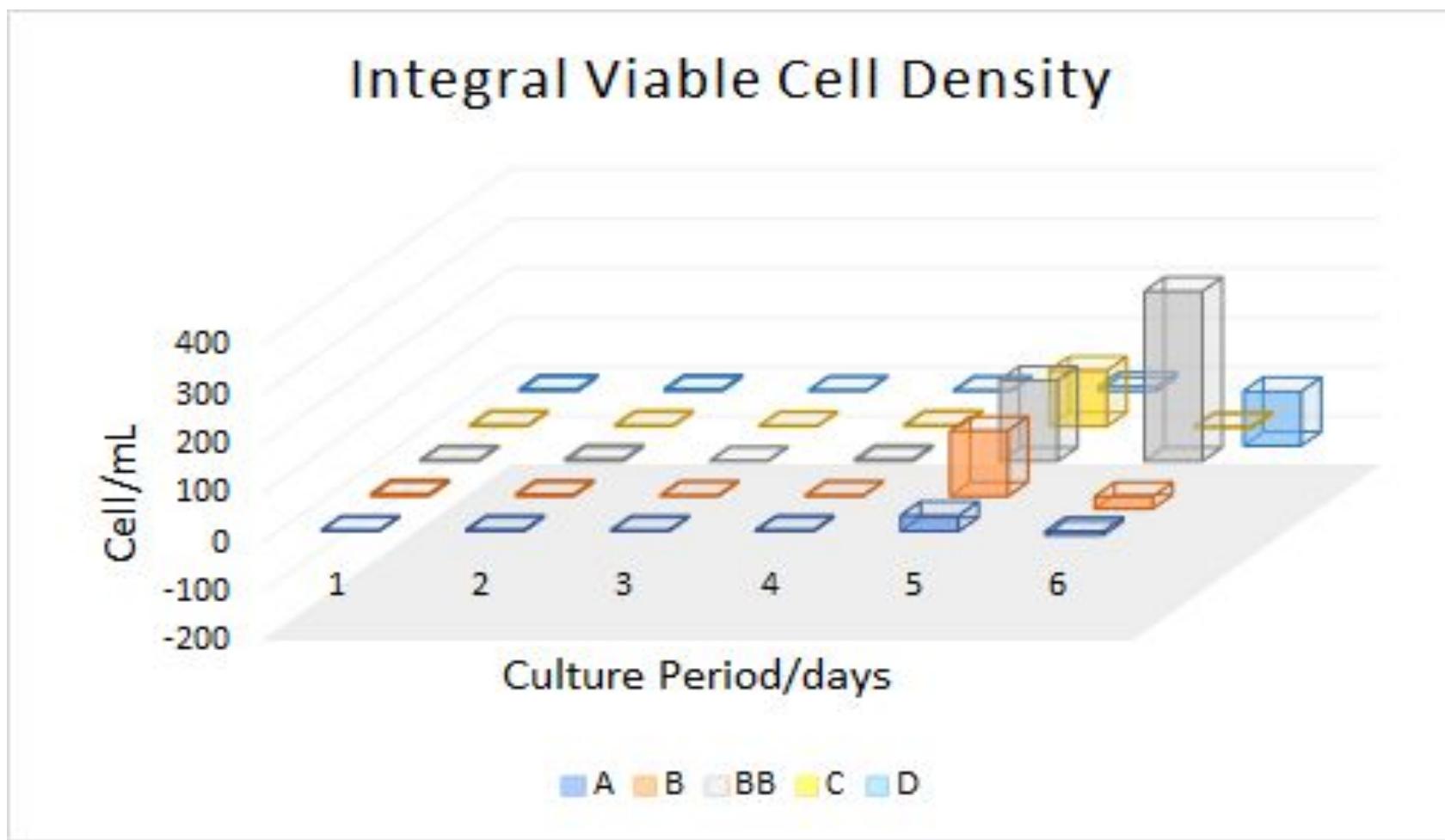


[1] Lin, H., Leighty, R. W., Godfrey, S., & Wang, S. B. (2017). Principles and approach to developing mammalian cell culture media for high cell density perfusion process leveraging established fed-batch media. *Biotechnology progress*, 33(4), 891-901.

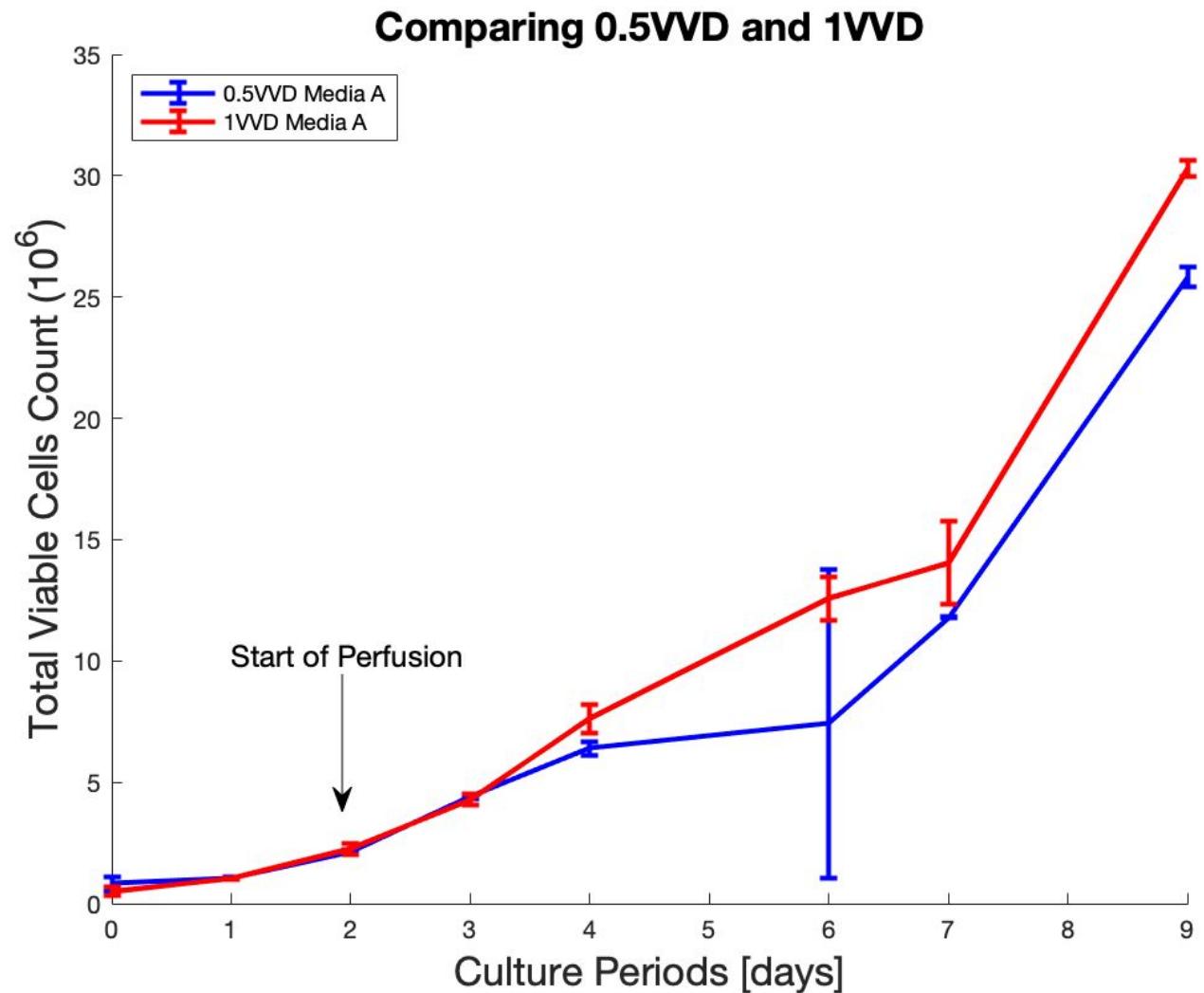
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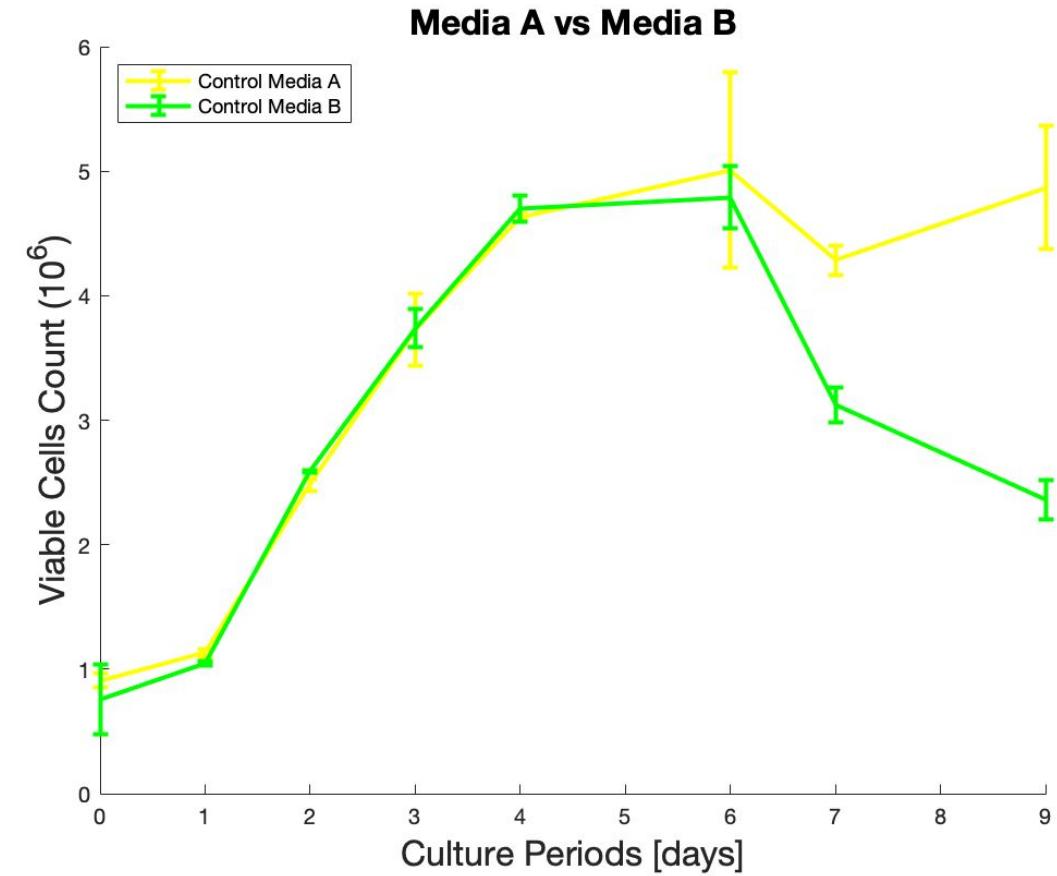
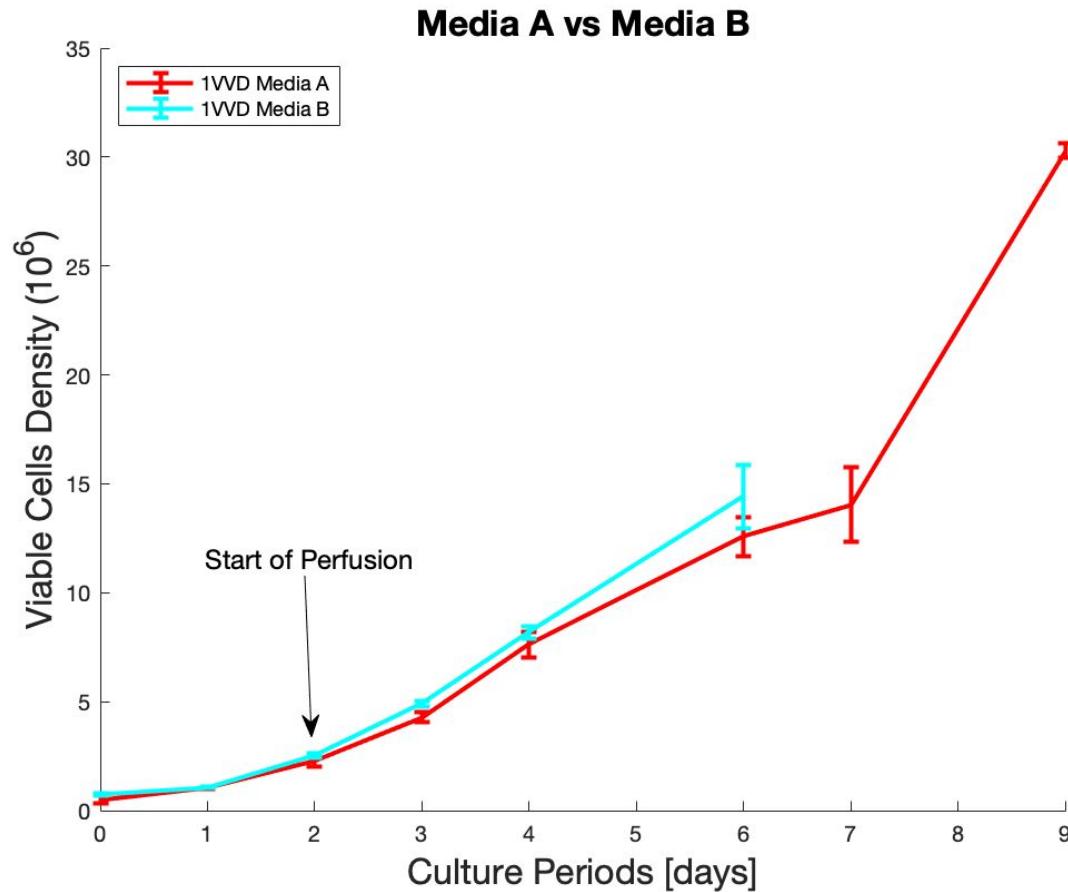
[1] Lin, H., Leighty, R. W., Godfrey, S., & Wang, S. B. (2017). Principles and approach to developing mammalian cell culture media for high cell density perfusion process leveraging established fed-batch media. *Biotechnology progress*, 33(4), 891-901.



## 0.5 VVD vs 1 VVD



# Media A vs Media B



# Results and Data Analysis

## Conclusion

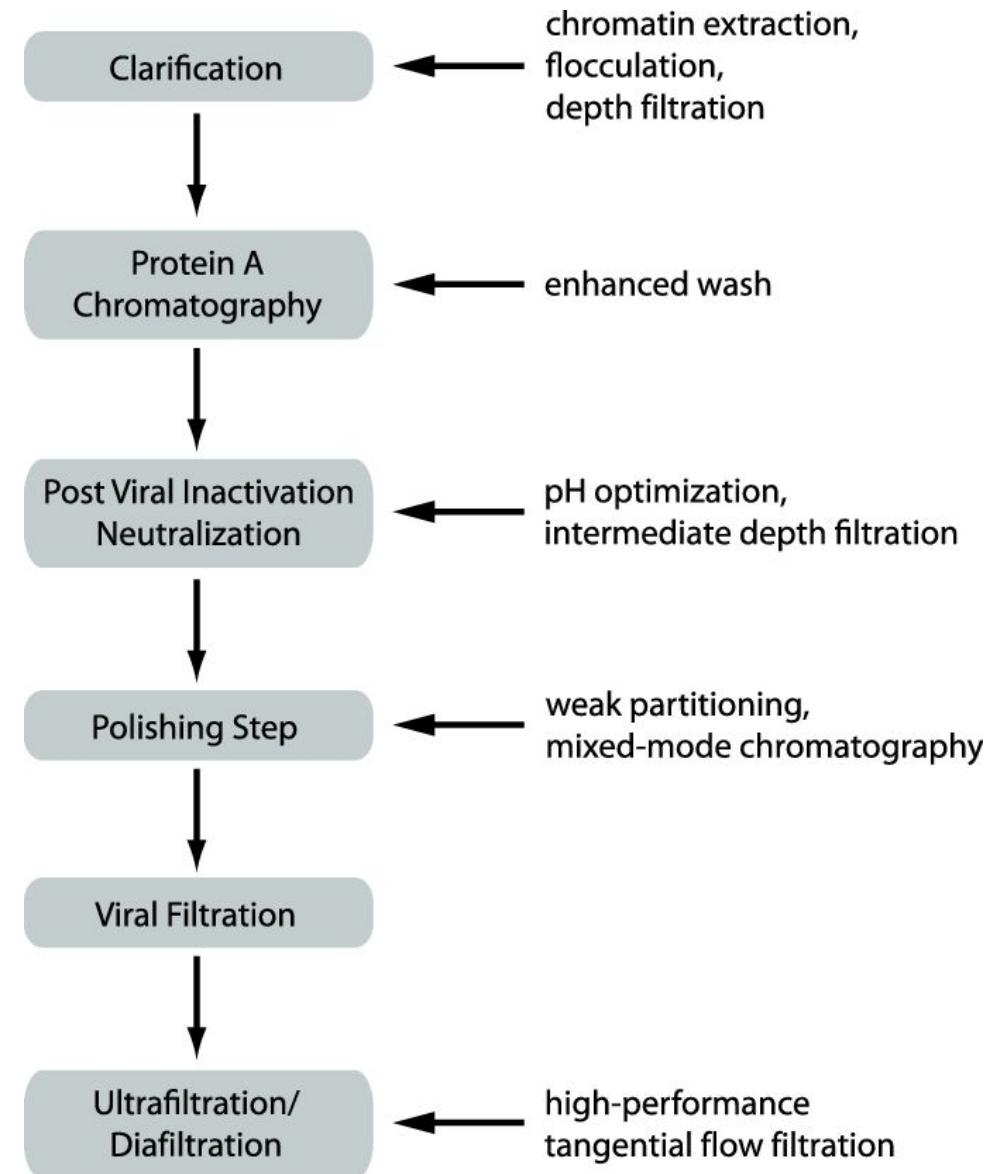
- Perfusion increases the viable cells density under both Media A and Media B conditions.
  - 1VVD has more significant effect on VCD and percent viability compared to 0.5 VVD and 0.5–1 VVD.
- The increase in cell numbers also increase the production of proteins. Thus, perfusion is beneficial to protein production.  
(<https://www.ncbi.nlm.nih.gov/pubmed/22159888>)
- This experiment is not significant enough to determine whether Media A or Media B is better culturing media. This might due to the fact that the cells densities are too low for anti-clump to be effective.
- Future improvement of this experiment: culture the cells for longer period of time; make sure all conditions start out with the same number of cells.



# Downstream

## Downstream Processing

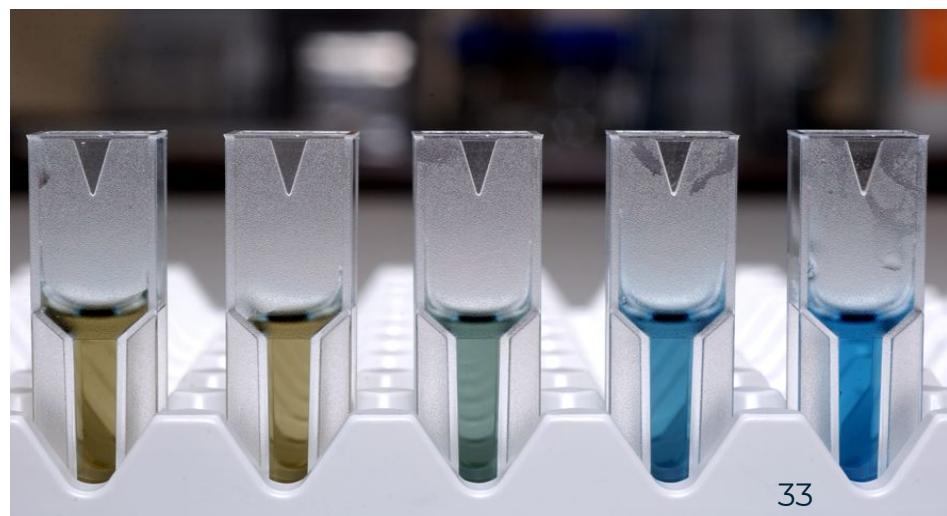
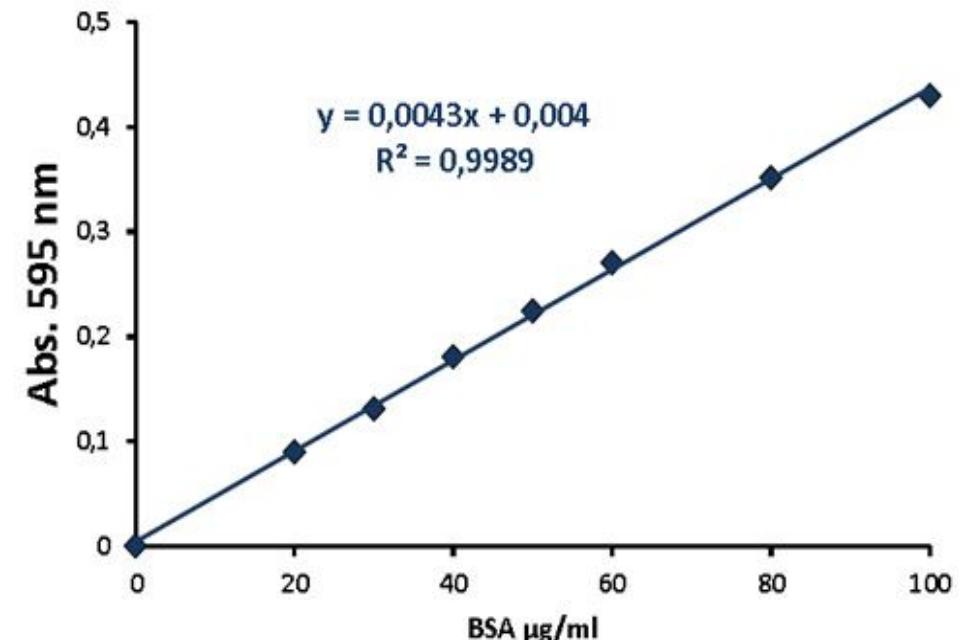
- Monoclonal Antibody purification
- Purpose: maximize product recovery and minimize production cost
- General steps:
  - Removal of Impurities
  - Viral removal
  - Product isolation
  - Product recovery



# Protein Concentration Determination

## Bradford Assay

- Purpose: Test that we optimized the upstream process by producing optimize MAP instead of waste product
- Determine the total protein concentration
- Spectrophotometer
- Bovine serum albumin
  - Protein
- Dye Coomassie Brilliant Blue G-250
  - Acidic: red, double protonated, 470nm
  - Neutral: green, single protonated, 650nm
  - Basic: blue, unprotonated, 595nm



# Material

## Bradford



PPE



BSA Stock Solution (1mg/mL)



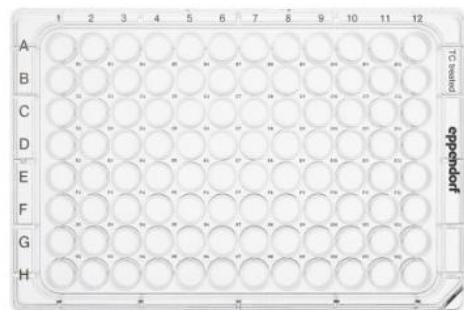
Protein Dye Reagent



MQ-H2O



50 mL Falcon tubes



Microtiter Plates

1.5 mL eppendorf tubes

Micropipettes

Tips



Unknown Samples



Microplate reader

# Method

## Bradford - BSA Standard Curve

Volume of Water (uL)	Protein Dye reagent volume (uL)	Volume of Stock Solution (V1 = uL)	Final Protein Concentration (C2 = ug/mL)
200	800	0	0
180	800	20	10
160	800	40	20
140	800	60	30
120	800	80	40
100	800	100	50
80	800	120	60
60	800	140	70

# Method

## Bradford and Blitz



Unknown  
Protein  
sample

Make 1x and 100X dilutions

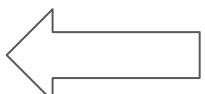


Add dye

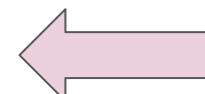
Vortex



BLITZ

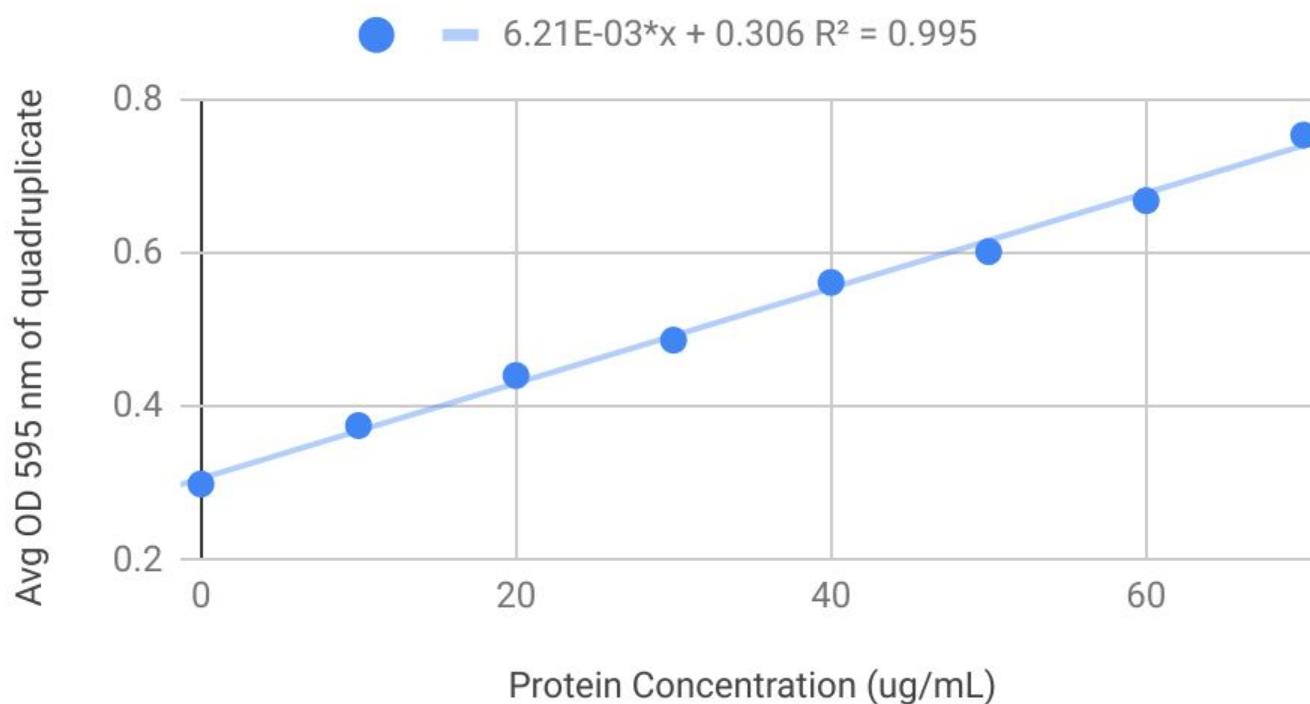


Microtiter Plate Reader



Microtiter Plate 36

BSA Standard Curve

**Unknown ID: M1062-1 (#3)**

$$y = 0.00621x + 0.306$$
$$R^2 = 0.995$$

Average Protein Concentration:  
1032.27 µg/mL

Blitz mAb Concentration: 863µg/mL

# Results

Sample ID	Blitz Concentration ( $\mu\text{g/mL}$ )	Total Protein Concentration ( $\mu\text{g/mL}$ )	% of Monoclonal Antibody
M10	863	1032.27	83.60%
M11	1728	2460	70.24%
M13	1347	2496.08	53.96%

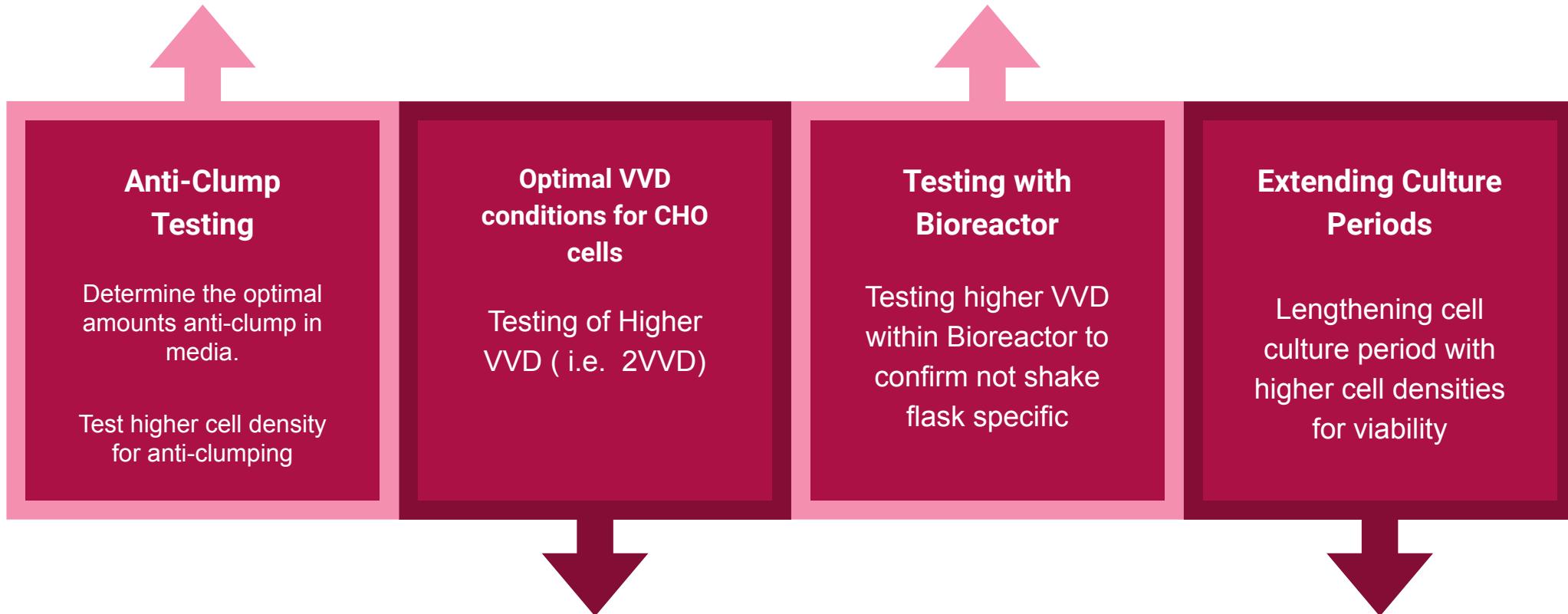
# Conclusion

- Downstream processing
- Bradford Assay and Blitz
- 3 unknown samples were analyzed and compared
- Sample M10 produces more mAb than M11 and M13



# Future Development

# Future Applications



# Takeaways

- ❖ Perfusion increases the viable cells density under both Media A and Media B conditions.
- ❖ More time would be needed to perform this experiment to determine whether Media A or B is better suited for culturing.
- ❖ Aseptic Technique is imperative for best possible results.
- ❖ Teamwork in a large group is important to coordinate for designation of tasks in and out of lab.

# Acknowledgements

## Mentors



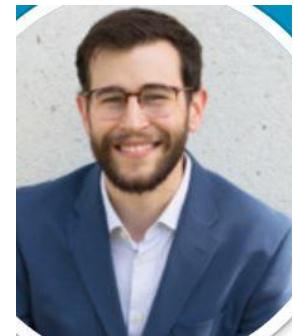
Dr. Raj Parti



Dr. Hu Zhang

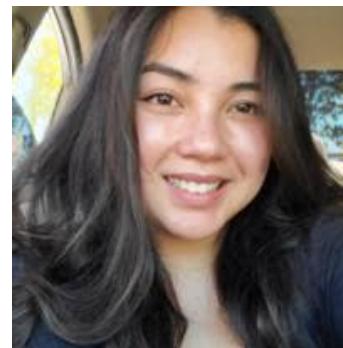


Dr. Corinna Doris



Dr. Andrew Burns

## Lab Support



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Christine Urrea



Aster Escalantes



Arneil Liban

# References

1. "Conversion of a CHO Cell Culture Process from Perfusion to Fed-Batch Technology without Altering Product Quality." *Journal of Biotechnology*, Elsevier, 30 Nov. 2005, [www.sciencedirect.com/science/article/pii/S0168165605006577](http://www.sciencedirect.com/science/article/pii/S0168165605006577).
2. Reinhart, David, et al. "Influence of Cell Culture Media and Feed Supplements on Cell Metabolism and Quality of IgG Produced in CHO-K1, CHO-S, and CHO-DG44." *SpringerLink*, BioMed Central, 14 Dec. 2015, link.springer.com/article/10.1186/1753-6561-9-S9-P36.
3. Altamirano, C., et al. "Improvement of CHO Cell Culture Medium Formulation: Simultaneous Substitution of Glucose and Glutamine." *Biotechnology Progress*, vol. 16, no. 1, 2000, pp. 69–75., doi:10.1021/bp990124j
4. "Responses of CHO Cell Lines to Increased pCO<sub>2</sub> at Normal (37 °C) and Reduced (33 °C) Culture Temperatures." *Journal of Biotechnology*, Elsevier, 18 Dec. 2015, [www.sciencedirect.com/science/article/pii/S0168165615302133?via=ihub](http://www.sciencedirect.com/science/article/pii/S0168165615302133?via=ihub).
5. Ha, Tae Kwang, and Gyun Min Lee. "Glutamine Substitution: the Role It Can Play to Enhance Therapeutic Protein Production." *Pharmaceutical Bioprocessing*, vol. 3, no. 3, 2015, pp. 249–261., doi:10.4155/pbp.15.6.
6. Zhu, Marie M., et al. "Effects of Elevated pCO<sub>2</sub> and Osmolality on Growth of CHO Cells and Production of Antibody-Fusion Protein B1: A Case Study - Zhu - 2005 - Biotechnology Progress - Wiley Online Library." *Biotechnology Progress*, American Chemical Society (ACS), 5 Sept. 2008, aiche.onlinelibrary.wiley.com/doi/full/10.1021/bp049815s.
7. Pan, Xiao, et al. "Selection of Chemically Defined Media for CHO Cell Fed-Batch Culture Processes." *Cytotechnology*, Springer Netherlands, Feb. 2017, [www.ncbi.nlm.nih.gov/pmc/articles/PMC5264622/](http://www.ncbi.nlm.nih.gov/pmc/articles/PMC5264622/).
8. Li, Feng, et al. "Cell Culture Processes for Monoclonal Antibody Production." *MAbs*, Landes Bioscience, 2010, [www.ncbi.nlm.nih.gov/pmc/articles/PMC2958569/](http://www.ncbi.nlm.nih.gov/pmc/articles/PMC2958569/).

# Appendix

