Investigation of the Effects of Critical Process Parameters such as Temperature, pH and pCO_2 on Charge Variants of mAbdi2

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1. INTRODUCTION

Charge variants in recombinant monoclonal antibodies (mAbs) are critical quality attributes (CQAs), which can affect function of antibodies in vitro and in vivo. Moreover, charge variants occur as cumulative effects of various post-translational modifications (PTMs) and chemical degradations on antibodies. These modifications can be occurred during both upstream and downstream processes. In the upstream process, critical process parameters (CPPs) such as temperature, pH and pCO_2 can be altered to ensure the desired product quality.

2. AIM

The subject of this poster is to investigate the effects of critical process parameters on the charge variants of mAbdi2, a biosimilar being developed at our biotechnology facility AbdiBio.

3. METHODS

- Recombinant Chinese Hamster Ovary (rCHO) cell line expressing the target monoclonal antibody was used in this study.

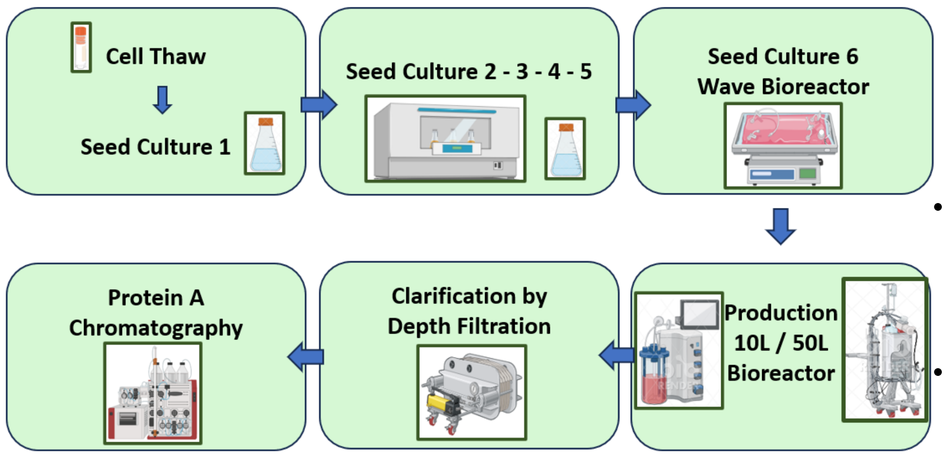
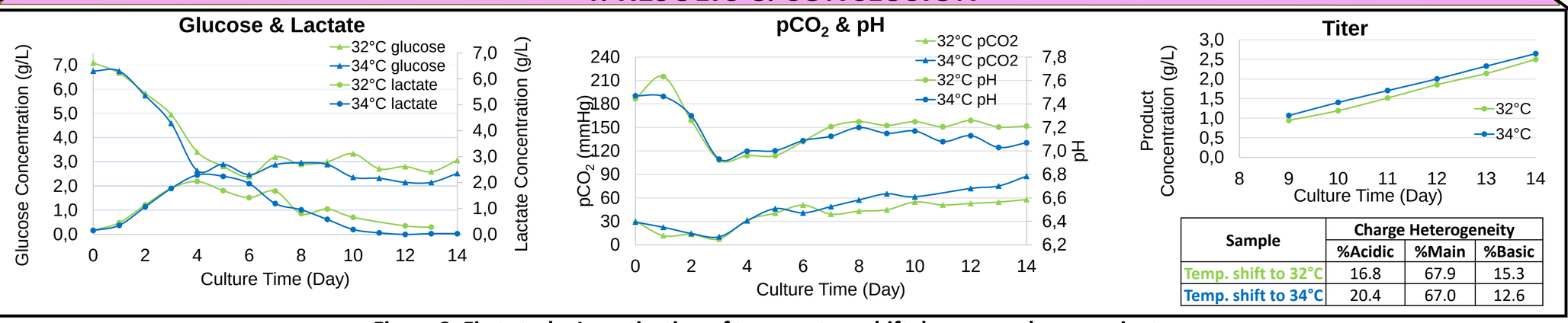


Figure 1. Process Flow Diagram

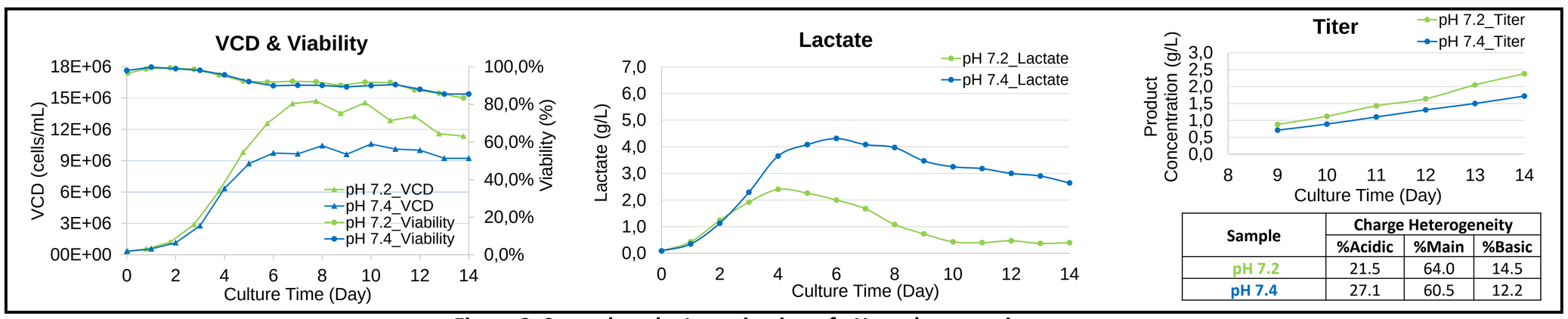
- In the first set of experiment, two parallel runs were performed to evaluate shift temperature effect: on the sixth day of production, operating temperatures at 37°C were reduced to 32°C and 34°C.
- In the second study, productions were carried out at pH set points of 7.2 and 7.4 to understand the effect of pH on charge variants.
- In the third set of experiment, effects of partial CO_2 pressure on change variants were observed. pCO_2 accumulation exceeding 120 mmHg (high) and controlling under 120 mmHg (low) throughout the production were compared.

4. RESULTS & CONCLUSION



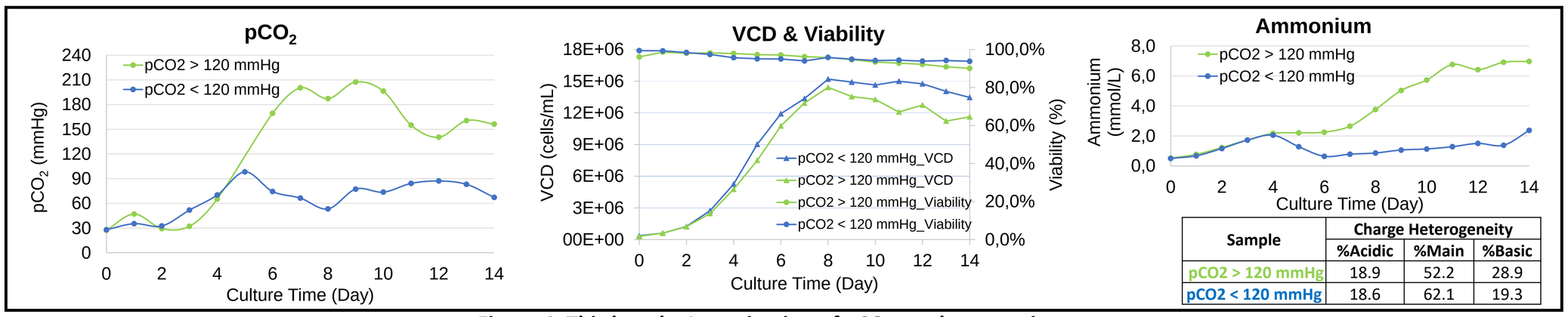
Sample	Charge Heterogeneity		
	%Acidic	%Main	%Basic
Temp. shift to 32°C	16.8	67.9	15.3
Temp. shift to 34°C	20.4	67.0	12.6

Figure 2. First study: Investigation of temperature shift degree on charge variants.



Sample	Charge Heterogeneity		
	%Acidic	%Main	%Basic
pH 7.2	21.5	64.0	14.5
pH 7.4	27.1	60.5	12.2

Figure 3. Second study: Investigation of pH on charge variants.



Sample	Charge Heterogeneity		
	%Acidic	%Main	%Basic
pCO2 > 120 mmHg	18.9	52.2	28.9
pCO2 < 120 mmHg	18.6	62.1	19.3

Figure 4. Third study: Investigation of pCO_2 on charge variants.

- In the first study where incubation temperature was shifted to 32°C and 34°C on the 6th day of production, lower percentage of acidic variants was observed in the product at lower shift temperature.
- When pH set points of 7.2 and 7.4 were compared, it was seen that as pH increased, the percentage of basic variants decreased whereas acidic variants increased in the product.
- Our last study showed that production at higher pCO_2 concentration resulted in an increase in the level of basic variants in the product.

5. DISCUSSION

- Lowering shift temperature decreased acidic and increased basic charge variant percentages in our candidate biosimilar product. Our findings align with those of Kishishita et al. (2015) and Sissolak et al. (2019).
- Irfan K. (2017) showed in his doctoral thesis that when pCO_2 accumulation in cell culture increases, the formation of basic variants also increases, consistent with our results.
- In accordance with Brunner et al.'s study (2016), our research demonstrated a decrease in acidic variants and a corresponding increase in basic variants at low pH values.

6. REFERENCES

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7. INFO

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