Sebastian Juan Reyes Davila, Michael Keebler***, Andreas Schulte****, Robert Voyer*, Yves Durocher*, Olivier Henry**, Phuong Lan Pham***

*Human Health Therapeutics Research Center, National Research Council Canada, 6100 Royalmount Avenue, Montreal , Quebec, H4P 2R2, Canada
**Polytechnique Montreal, 2500 Chem. de Polytechnique, Montréal, Quebec, H3T 1J4, Canada
***Kuhner Shaker Inc., 1160 Industrial Rd, Unit #8 San Carlos, CA 94070, USA
****Kuhner Shaker GmbH, Technologiepark Herzogenrath, Kaiserstraße 100, 52134 Herzogenrath, Germany

IMPACT OF PROTEIN PRODUCTION ON METABOLIC ACTIVITY OF CHO STABLE CELL LINE PRODUCING PALIVIZUMAB

Introduction

Shake flasks are an important first tool of mammalian cell process development. Despite them being mostly studied in an offline fashion (discontinuous measurement of key values), recent technological advances have allowed for the online monitoring of respiration rates:

- **Oxygen Transfer Rate (OTR) Importance:** Growth and metabolic function of mammalian cells are directly linked to their respiratory activity which can be estimated by measuring changes in the concentration of O_2 inside a vessel [1, 2].
- **Transfer rate Online Measurement (TOM):** The device **c**an monitor respiratory activity in a similar way to the Respiration Activity Monitoring System (RAMOS) [3].
- **Specific Oxygen Consumption Rates (qO_2):** Correlate with TCA cycle activity and thus protein production [4].
- **Process Related Conditions:** Can modulate specific respiration rates during a production process [5]. Within the context of this poster the impact of protein production on OTR profiles will be studied.

Experimental Set-up

Inducible CHO cell line expressing palivizumab

Fed-Batch Process

Offline Measurements:
- Viable and Total Cell Density
- Viability
- Glucose
- Lactate
- Ammonia
- Palivizumab titer
- Cell diameter

Condition
- Induction [n1]
- Induction [n2]
- Induction [n3]
- No-Induction [n1]
- No-Induction [n2]
- No-Induction [n3]

Temp. Shift from 37°C to 32°C (Dpi)	Seeding Density (x10^6 cells/mL)	Cumate Induction	Orbit (mm)	RPM	Flask Size
2	0.4	Yes	25	200	250 mL
2	0.4	Yes	25	200	250 mL
2	0.4	Yes	25	200	250 mL
2	0.4	No	25	200	250 mL
2	0.4	No	25	200	250 mL
2	0.4	No	25	200	250 mL

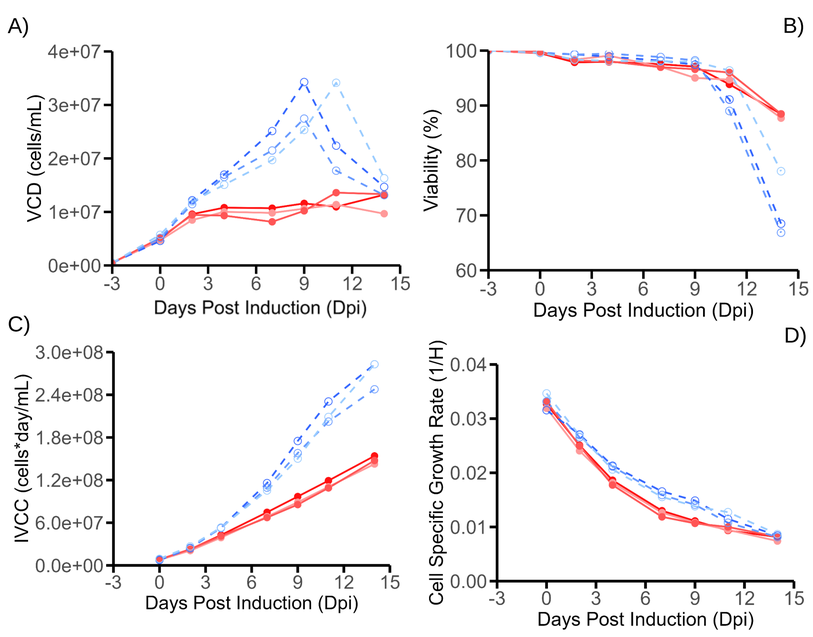

Figure 1. Induction Impact on Growth Profiles

Induction impact on A) Viable Cell Density (VCD) B) Viability C) Integral of viable cell concentration (IVCC) D) Growth rate.

- **Advent of cumate induction slows down cell growth before temperature shift (0-2 Dpi)**
- **Non-induced cultures continue growing despite temperature reduction at 2 Dpi**
- **Non-induced cultures decline after 9 dpi (day 12) as feed regimen was not adapted to sustain 3-fold increase in peak VCD**

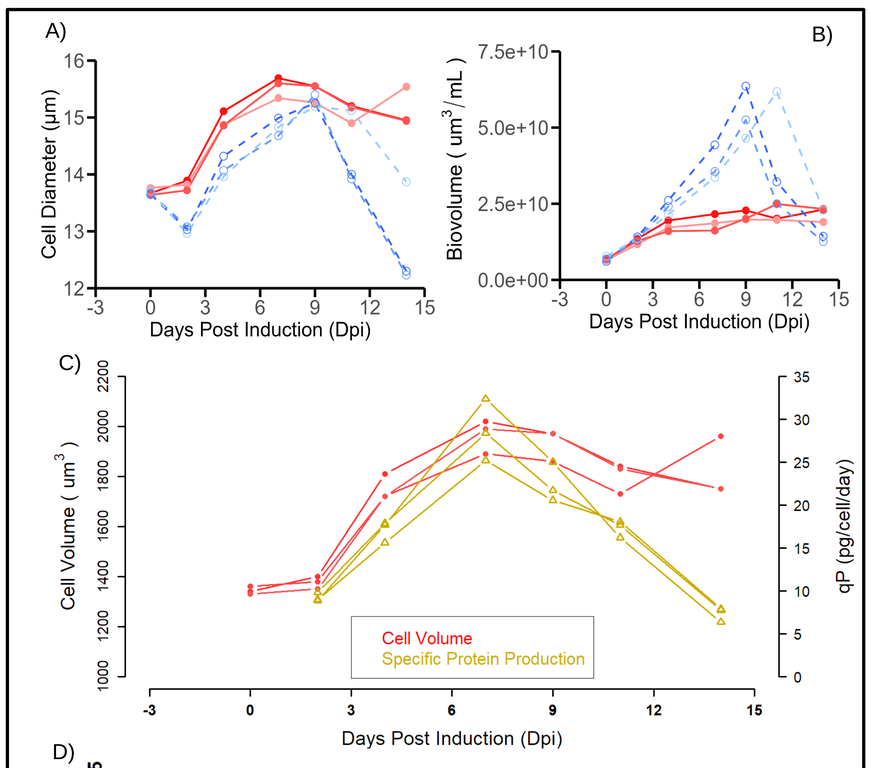

Figure 2. Induction Impact on Cell Volume

A) Cell diameter B) Biovolume C) Cell volume and cell specific protein productivity (qP) scatterplot D) qP and cell volume scatterplot.

- **Cell swell across culture time but more so with the addition of cumate.**
- **Biovolume shows sharp decrease faster than that of VCD given that average cellular diameter shrinks during the decline phase.**
- **After induction, the increase in cell volume correlates strongly with specific protein production.**
- **Scatter plot suggests that there is a relationship between increasing cell volume and increasing productivity up to 11 dpi (R^2= 0.76).**

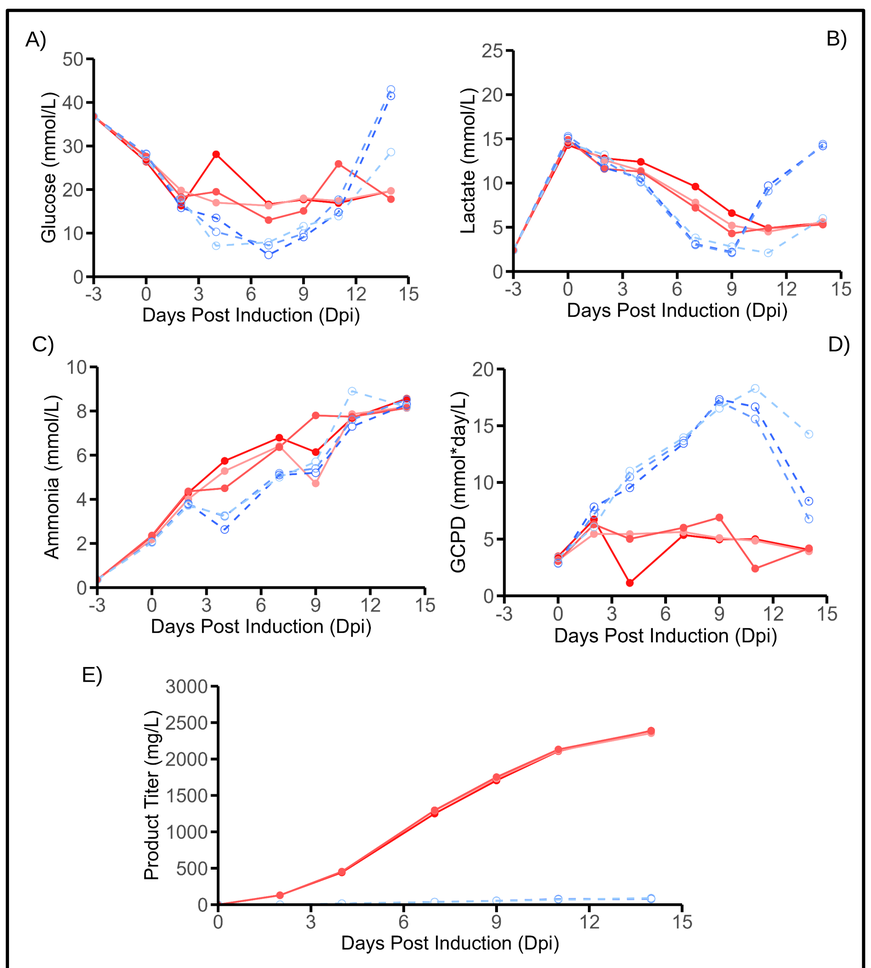

Figure 3. Induction Impact on Volumetric Metabolic Activity

A) Residual glucose B) Lactate profile C) Ammonia profile D) Glucose consumed per day (GCPD) E) Titer profile

- **Non-induced cells consume more glucose and lactate than induced cells.**
- **Lactate was consumed between Day 3 and day 5 (begins at 0 Dpi in case of induction) regardless of induction status.**
- **Ammonia accumulation for non-induced cells is less between 0 Dpi and 9 Dpi despite such condition having higher VCD.**
- **Protein production leakage without cumate is approx. 4%.**

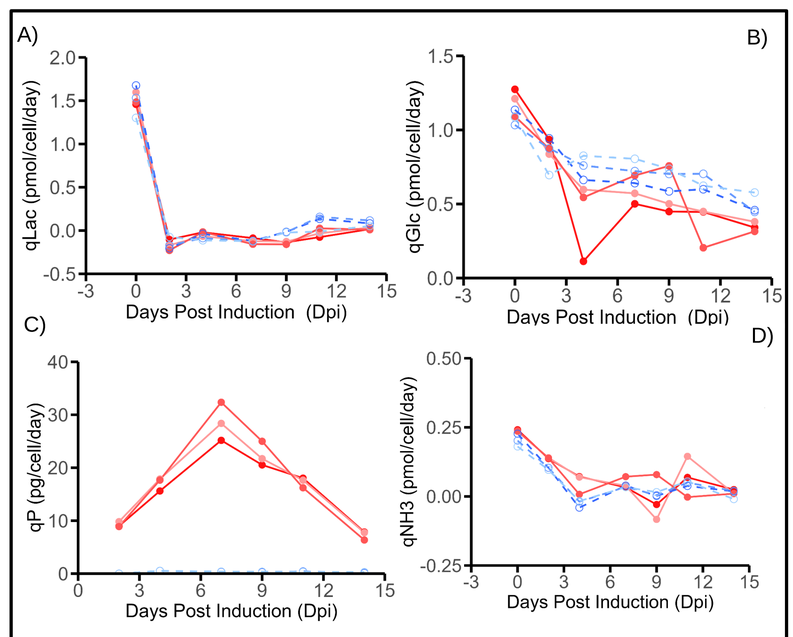

Figure 4. Induction Impact on Specific Metabolic Activity

A) Specific lactate production rate (qLac) B) Specific glucose consumption rate (qGlc) C) Specific protein production rate (qP) D) Specific ammonia production rate (qNH_3)

- **Specific lactate production rate differs only between 9-14 Dpi where the decline phase drives an increase in lactate accumulation.**
- **Specific glucose consumption remains higher in non-induced cultures between 2-14 Dpi.**
- **Specific ammonia production is lower in non-induced cultures between 2-7 Dpi.**
- **Peak specific protein production is 55-fold higher in induced cultures when compared to non-induced cultures.**

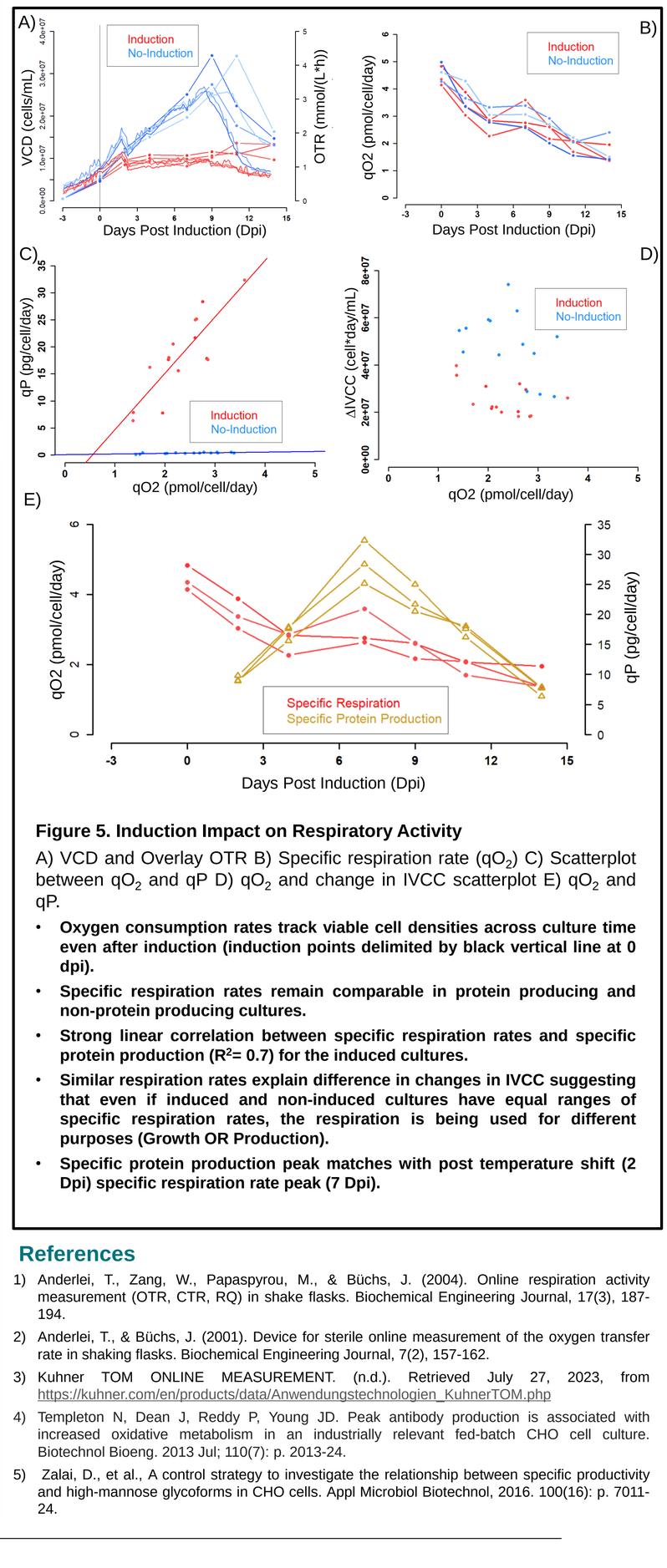

Figure 5. Induction Impact on Respiratory Activity

A) VCD and Overlay OTR B) Specific respiration rate (qO_2) C) Scatterplot between qO_2 and qP D) qO_2 and change in IVCC scatterplot E) qO_2 and qP.

- **Oxygen consumption rates track viable cell densities across culture time even after induction (induction points delimited by black vertical line at 0 dpi).**
- **Specific respiration rates remain comparable in protein producing and non-protein producing cultures.**
- **Strong linear correlation between specific respiration rates and specific protein production (R^2= 0.7) for the induced cultures.**
- **Similar respiration rates explain difference in changes in IVCC suggesting that even if induced and non-induced cultures have equal ranges of specific respiration rates, the respiration is being used for different purposes (Growth OR Production).**
- **Specific protein production peak matches with post temperature shift (2 Dpi) specific respiration rate peak (7 Dpi).**

References

1) Anderlei, T., Zang, W., Papaspyrou, M., & Büchs, J. (2004). Online respiration activity measurement (OTR, CTR, RQ) in shake flasks. Biochemical Engineering Journal, 17(3), 187-194.
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