PA 123

Effect of elevated carbon dioxide concentration on IgG production using CHL-YN cells

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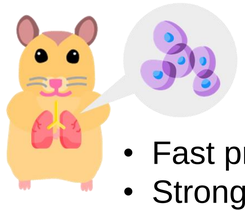
Introduction

Chinese hamster lung (CHL)-YN cells[1]

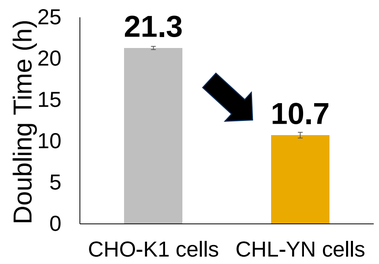


A novel cell line derived from primary cultures of Chinese hamster lung tissue

- Fast proliferation
- Strong glutamine synthetase
- Similar glycosylation patterns to IgG_1 produced in CHO cells

CHL-YN cells have a potential to be important expression host cells for increasing productivity in manufacturing of biopharmaceuticals.

Comparison of doubling time between CHL-YN cells and CHO-K1 cells



The doubling time of CHL-YN cells is twice as short as Chinese hamster ovary (CHO)-K1 cells.

The importance of carbon dioxide (CO_2) in cell culture[2]

1. pH control: CO_2-Bicarbonate based buffer system 2. By-product of cell metabolism

Challenge

In a large scale or high cell concentration, CO_2 can be accumulated throughout the process if left uncontrolled.

Previous study: Effects of elevated CO_2 concentrations in CHO cells [3]

Inhibit cell growth

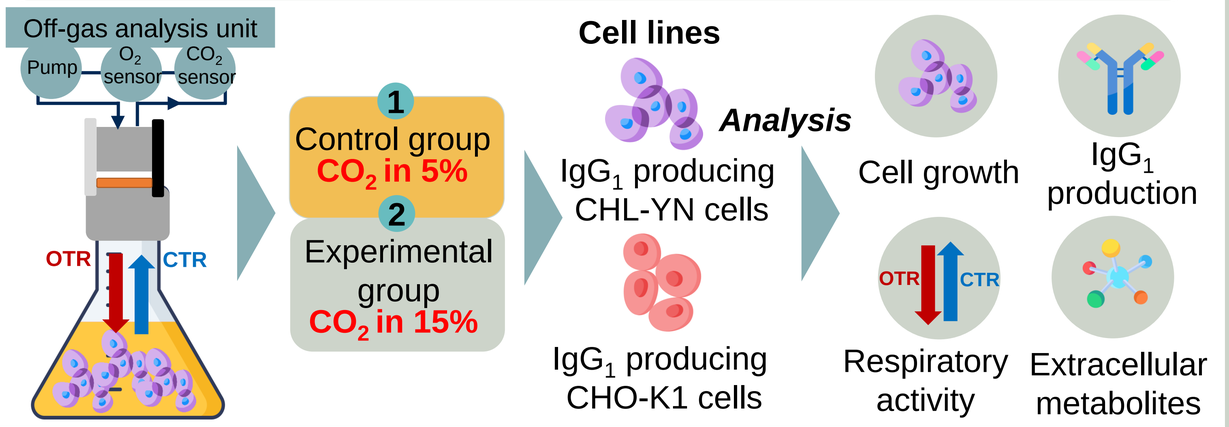
Reduce titer of IgG

Undesired quality of IgG

CO_2 concentrations **≈16%-27%** have been reported to inhibit growth and IgG production[3].

Objective

To investigate the effect of elevated CO_2 concentration on IgG_1 production produced by CHL-YN cells compared to CHO-K1 cells, focusing on 5% CO_2 concentration and the elevated CO_2 concentration at 15%.



Materials and Methods

Culture condition

- Batch cultivation
- EX-CELL® CD CHO
- Fusion medium
- 500 mL Erlenmeyer flask
- Temperature:37 °C
- Speed: 140 rpm
- Aeration rate: 16 mL/min
- Measurement phase: 20 mins
- Aeration phase: 60 mins

Online measurement

Oxygen Transfer Rate (OTR) and Carbon dioxide Transfer Rate (CTR)

→ The Kuhner TOM (Transfer-Rate Online Measurement)

Measure online in flask via non-invasive and head space analysis

qO_2 calculation from OTR[4]

$$\frac{dc_{O_{2,l}}}{dt} = OTR - OUR$$

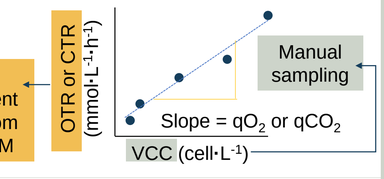
$$OUR = OTR = \boldsymbol{q_{O_2}} x\, VCC$$

qCO_2 calculation from CTR

$$c_{CO_{2,l}} = \frac{CTR}{K_L a_{CO_2}} + c^*_{CO_2}$$ [5]

$$CER = CTR = \boldsymbol{qCO_2}\, x\, VCC$$

Online measurement obtained from Kuhner TOM



Offline measurement

Cell concentration
→ Vi-CELL™ XR cell viability analyzer

IgG_1 concentration
→ ELISA assay

Extracellular metabolites concentration
→ YSI 2950 Biochemistry analyzer

Osmolality
→ Bioprofile FLEX2

pH
→ LAQUA pH METER F-71

Conclusions

- IgG_1 producing CHL-YN cells and IgG_1 producing CHO-K1 cells responded differently to elevated CO_2 concentration at 15% in terms of cell growth, respiration activity, and extracellular metabolite concentration, especially IgG_1 production indicating, that the responses depended on cell types.
- **The elevated CO_2 concentration at 15% enhanced IgG1 production in IgG_1 producing CHL-YN cells but inhibited cell growth in both cell lines.** Furthermore, increasing CO_2 concentration increased the demand for glucose consumption while lowering lactate production and glutamine consumption. It also led to a drop in demand for O_2 consuming while raising CO_2 production.

Results and Discussion

1. Cell growth analysis

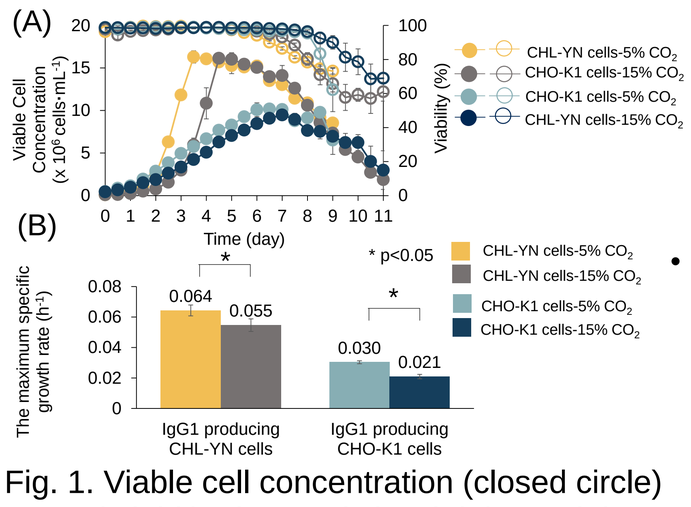


Fig. 1. Viable cell concentration (closed circle) and viability (opened circle) (A) μ_{max} (B)

- Elevated CO_2 concentration from 5% to 15% decreased the maximum specific growth rate (μ_{max}) 15% in IgG_1 producing CHL-YN cells and 31% in IgG_1 producing CHO-K1 cells.
- IgG_1 producing CHL-YN cells showed a lower percentage decrease in μ_{max} compared with IgG_1 producing CHO-K1 cells, suggesting they might be less susceptible to elevated CO_2 concentration.

2. IgG_1 production analysis

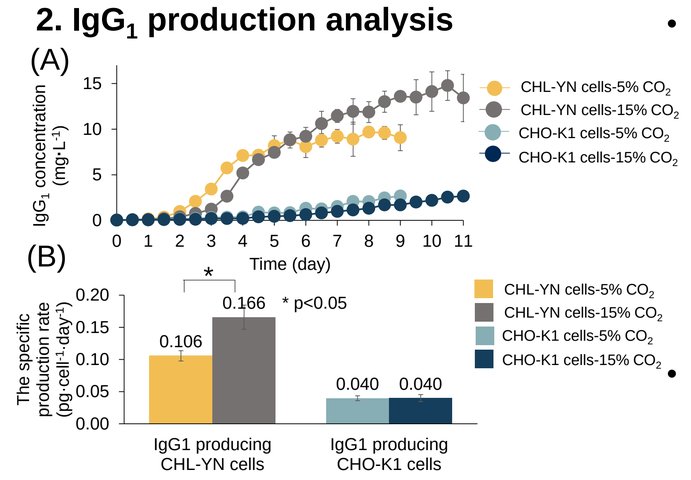


Fig. 2. IgG_1 concentration (A) q_{mAb} (B)

- The specific production rate (q_{mAb}) increased 57% at 15% CO_2 compared with the control. IgG_1 concentration started increasing greater than the control from day 5, resulting in raising final IgG_1 titer in IgG_1 producing CHL-YN cells.
- In contrast, the q_{mAb} and final IgG_1 titer of IgG_1 producing CHO-K1 cells at 15% CO_2 were similar to the control.

3. Respiratory activity analysis

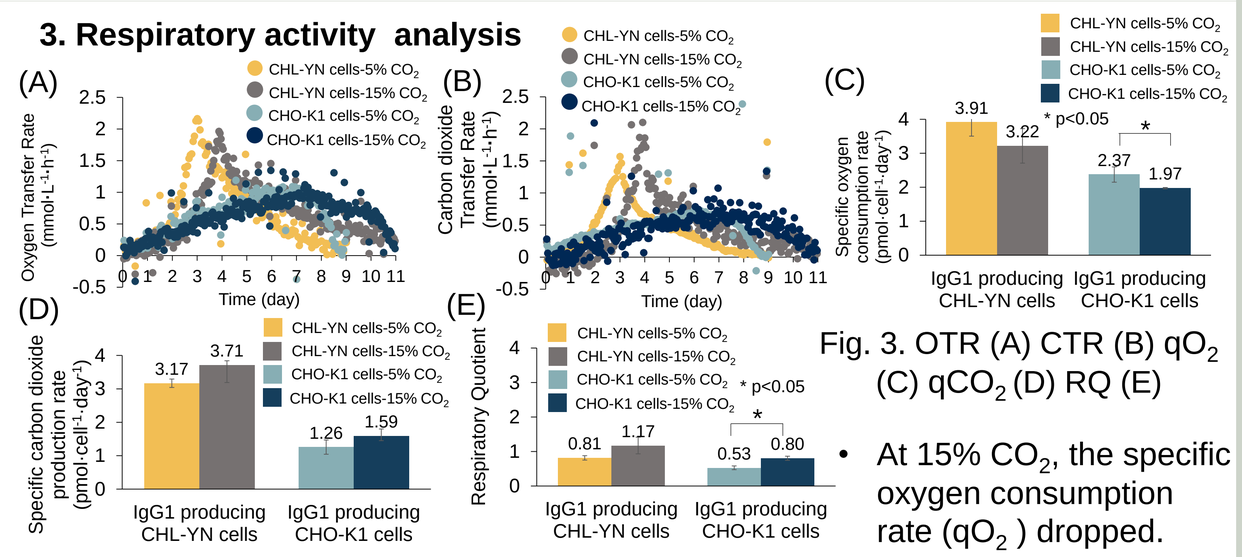
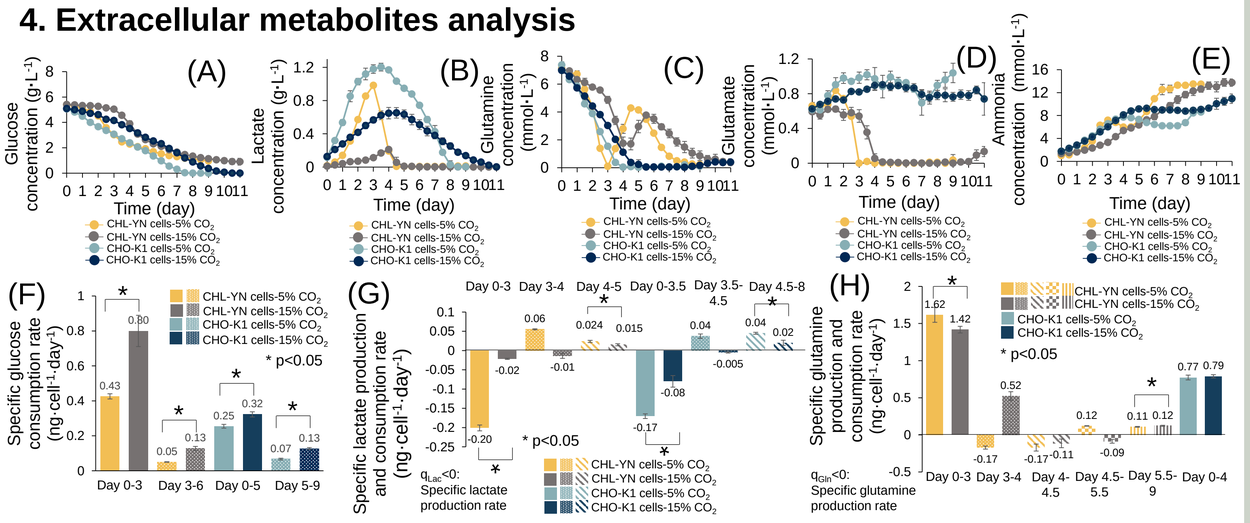


Fig. 3. OTR (A) CTR (B) qO_2 (C) qCO_2 (D) RQ (E)

- At 15% CO_2, the specific oxygen consumption rate (qO_2) dropped.
- While the specific carbon dioxide production rate (qCO_2) raised in both cell lines, consequently the respiratory quotient (RQ ($\frac{qCO_2}{qO_2}$)) increased.
- IgG_1 producing CHL-YN cells showed greater qO_2 and qCO_2 than IgG_1 producing CHO-K1 cells even at elevated CO_2 concentration, indicating they grew faster, demand for consuming O_2 and producing CO_2 increased.

4. Extracellular metabolites analysis



- At 15% CO_2, the specific glucose consumption rate (q_{Gluc}) increased in IgG_1 producing CHL-YN cells. The specific lactate production rate (q_{Lac}) decreased and lactate shift was delayed in both cell lines. The specific glutamine consumption rate (q_{Gln}) decreased in IgG_1 producing CHL-YN cells.

Fig. 4. Extracellular metabolite concentration: Glucose (A) Lactate (B) Glutamine (C) Glutamate (D) Ammonia (E) q_{Gluc} (F) q_{Lac}(G) q_{Gln}(H)

5. pH and osmolality

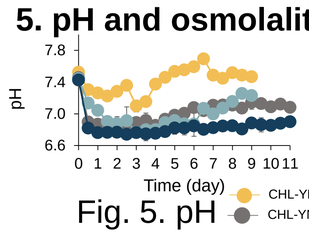


Fig. 5. pH

- The increase in CO_2 concentration may lead to increase dissolved CO_2, subsequently raising H_2CO_3 concentration and lowering pH.
- The osmolality at elevated CO_2 concentration slightly increased in both cell lines.

References

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Acknowledgements

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