Oxygen Transport Phenomena in Single-Use Shaking Flasks under Reduced Oxygen Conditions

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1. Objective

The oxygen concentration in cultivation media can have a significant effect on the proliferation and metabolism of stem cells and *Chinease Hamster Ovary* (CHO) cells [1, 2]. The aim of this work was to characterize mass transfer phenomena of shaking flasks with atmospheric and reduced oxygen conditions.

2. Kuhner LT-XC

To control the oxygen concentration during shaking flask cultivations, a novel shaker (Kuhner Shaker; LT-XC with O_2 control) was developed by the Kühner AG. It controls:

- Temperature
- Humidity
- Carbon dioxide concentration
- Oxygen concentration
- Shaking frequency

Figure 1 Kuhner LT-X, LT-XC has a similar design

3. Methods

A disposable baffled Erlenmeyer flask (working volume: 80 mL, Corning) with a vent cap (0.2 µm) was equipped with a dissolved oxygen sensor and valves, in order to flush the shaking flask with nitrogen or pressured air. The mass transport was described by the volumetric mass transfer coefficient (1) from the headspace of the shaking flask ($pO_{2,head}$) into the liquid (dissolved oxygen: DO) and (2) from the shaker ($pO_{2,shaker}$) through the headspace into the liquid. All experiments were performed based on the DECHEMA guideline “Recommendations for process engineering characterization of single-use bioreactors and mixing systems by using experimental methods” [3]

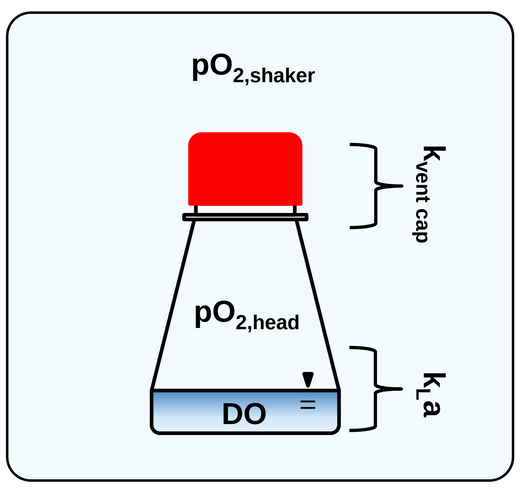

Figure 2 Mass transfer in shaking flask systems

Oxygen transfer

$$\frac{dc}{dt} = k_{vent\ cap}\,[or]\,k_La \cdot (c_L{}^* - c_L)$$

Simulation of oxygen transfer

$$c(t) = (1 - c_L^*) \cdot e^{-k_{vent\ cap}\,[or]\,k_La \cdot t} + c_L^*$$

c_L	bulk concentration liquid
$c_L{}^*$	saturation concentration liquid
t	time

4. Results and Discussion

As can be seen in Figure 3, the k_La is constant for shaking rates between 20 – 80 min^{-1}, which is suitable for the cultivation of stem cells on microcarriers. For shaking rates between 80 – 250 min^{-1}, the k_La rises up to 118 h^{-1}, which is applicable for CHO cultivation. A further increase of the shaking rate leads to a collapse of the fluid movement and to a reduced mass transfer.

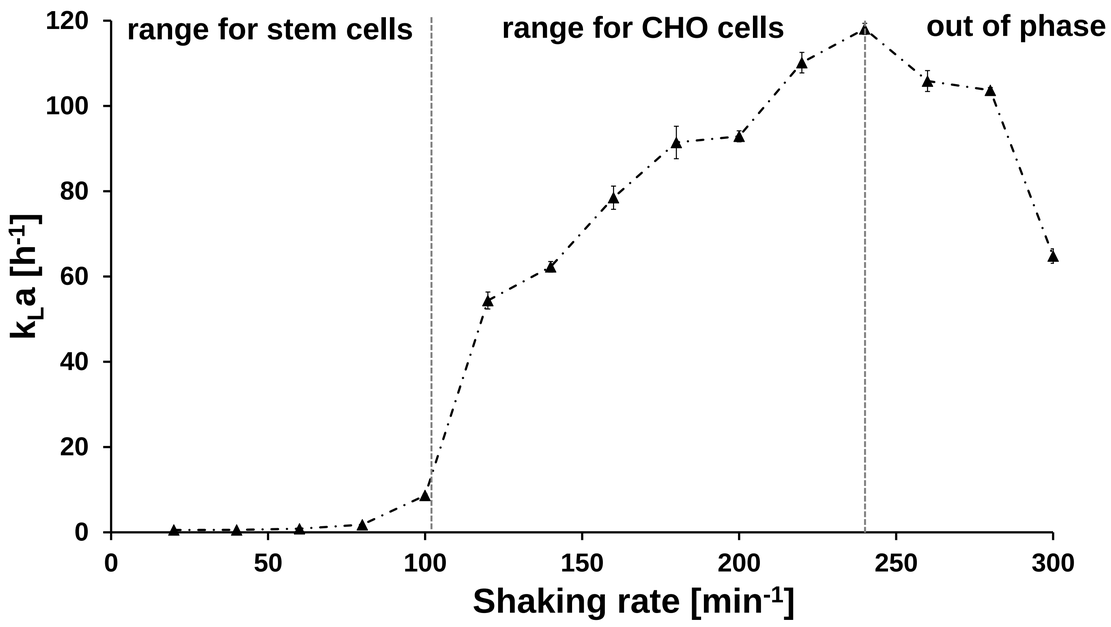

Figure 3 k_La in comparison to the shaking rate, 85 % Humidity, 37°C, 20 % pO_2, V = 80 mL (10 g L^{-1} NaCl in water), head space gassing with air was applied, 12.5 mm shaking diameter

The mass transfer coefficient $k_{vent\ cap}$ was determined to be **3.8 h^{-1},** which is lower than the k_La and is regarded as the limiting mass transfer resistance during medium saturation, e.g. after sampling. The simulated time until 90 % of the adjusted DO (set-point shaker) was reached is 60 min (Figure 4).

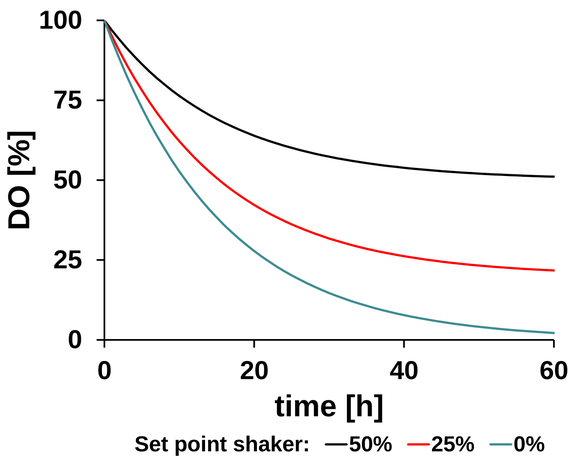

Figure 4 Simulated dissolved oxygen concentration, experimentally verified (not shown)

Even if the gas composition inside the shaker has readjusted quickly, **a slow decrease of the oxygen level inside the shaking flask after sampling was observed**. Depending on the cell line, this may lead to oxidative stress.

5. Outlook

In the future, CHO cells and stem cells (e.g. Figure 5) will be cultivated under reduced oxygen concentrations in shaking flasks. The effect of oxygen on the growth and metabolism will be evaluated.

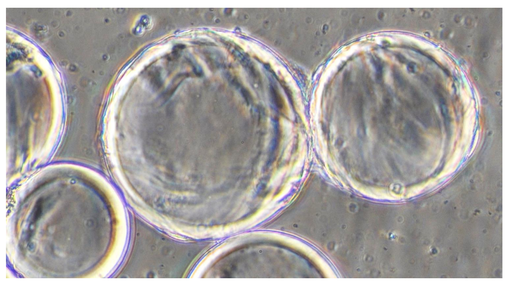

Figure 5 Sweat gland-derived stem cells, Cytodex 3 microcarrier, DMEM (4.5 g L^{-1} D-glucose), 0.1 g L^{-1} FGF-2, 10 % human serum, 5 % CO_2, 5 % O_2, V = 40 mL, 80 min^{-1} shaking rate, 12.5 mm shaking diameter, Kuhner LT-XC

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References

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