Mammalian Cell Cultivation using AerationCaps for Shake Flask Gas Supply

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Objective

Mammalian cell cultivation using shake flasks requires agitation in CO_2 enriched atmosphere for pH control [1, 2]. To perform cultivations in rather simple shakers, e.g. only temperature and shaking control, AerationCaps (Kühner AG) are an add-on device to supply and maintain a controlled gas atmosphere needed for mammalian cell culture. Therefore, the applicability of AerationCaps is investigated in this poster for mammalian cell culture processes.

Aeration setup

A scheme of the Kuhner AerationCap mounted on a shake flask is shown in Figure 1. The AerationCap has two luer-lock connectors to allow the gas supply. To prevent air from the surroundings entering into the flask, the AerationCap has a rubber sealing on the bottom. The caps can be easily installed on the shake flask and removed as needed, without any additional modifications to the flask. In conjunction with a controlled gas supply (i.e., FlowCon), the caps can be used to ensure the desired atmosphere inside the flasks, regardless of the incubator type.

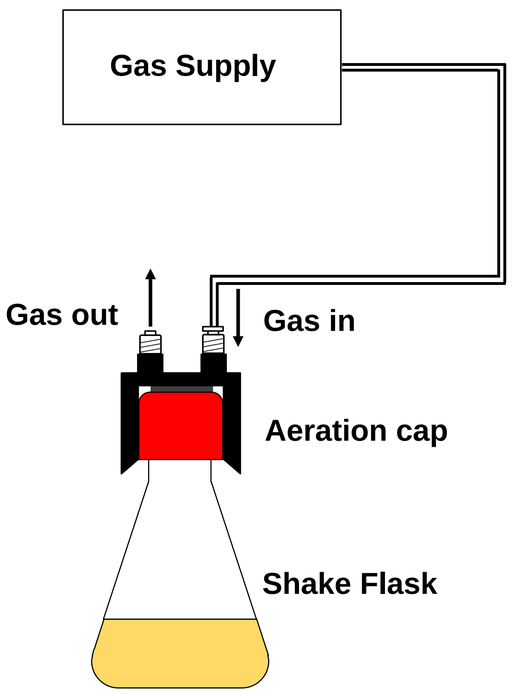
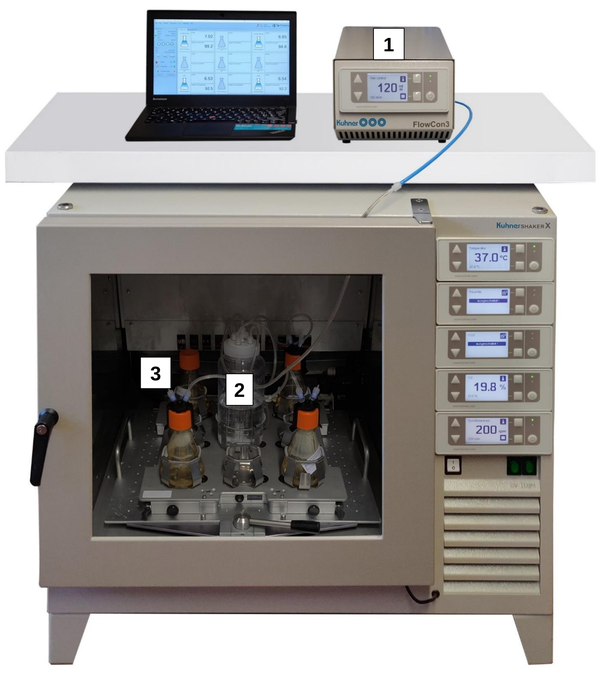


Figure 1 Schematic of shake flask with AerationCap connected to gas supply by luer-lock attachments

To control the composition of the gas supply, Kühner AG offers FlowCon, a gas mixing device with up to four inputs and an adjustable output flow rate.

The output of the gas mixing-device can be equilibrated, humidified and divided between four flasks using the Kuhner GasDivider. An exemplary set-up of the aeration system is shown in Figure 2.



1. FlowCon
2. GasDivider
3. AerationCap

Figure 2 Setup with AerationCap, GasDivider and FlowCon

Case Study: pH buffering in cell culture

CHO DP-12 cells were cultivated using AerationCaps (n=2) and FlowCon (flow rate: 120 ml min^{-1}) to maintain 5% CO_2 for pH buffering and humidity control. No aeration or humidity control was adjusted using the incubator controls. Cells from the same preculture were cultivated in a reference incubator with 5% CO_2 control (n=2). The Shake Flask Reader (SFR, PreSens) was used with the LT-X shaker (Kuhner AG) for non-invasive, online monitoring of pH and dissolved oxygen (DO) in the shake flasks.

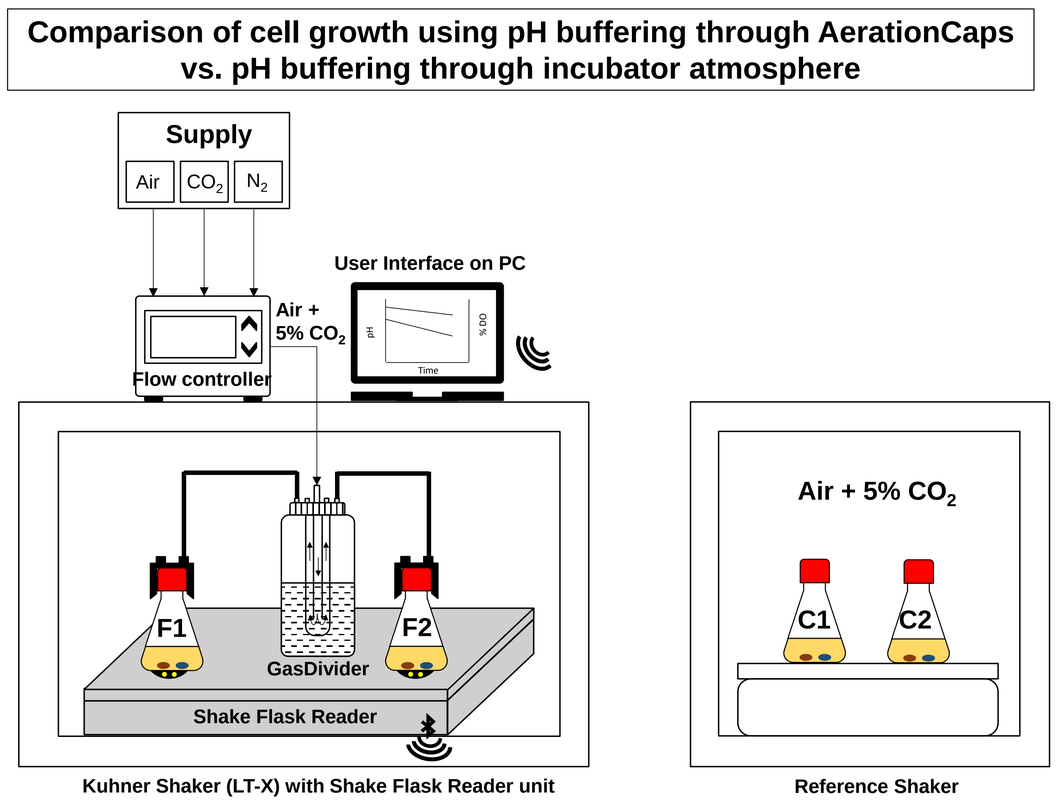


Figure 3 Cultivation of CHO DP-12 cells using aeration cap and FlowCon3 to maintain 5% CO_2 Flask volume: 80 ml, working volume: 40 ml, medium: TC-42 (Xell AG) with 6 mmol l^{-1} glutamine

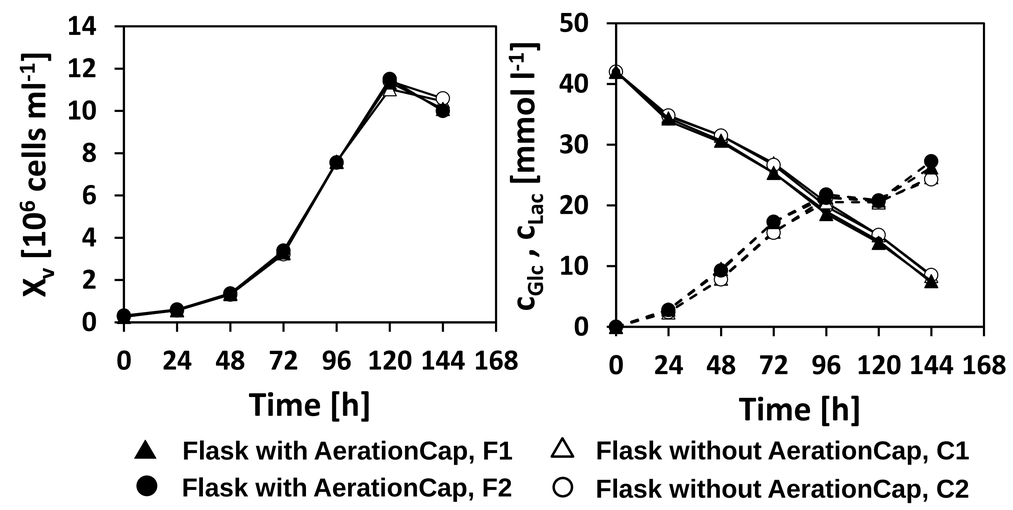


Figure 4 Experimental results of CHO DP-12 cultivations with and without AerationCaps; A: Cell growth, B: Glucose (solid line) and Lactate (dashed line) concentrations

- ✓ Using AerationCaps, **cell growth and metabolic activity are comparable** to the cell growth and metabolic activity at incubator atmosphere with 5% CO_2

Conclusion

FlowCon with AerationCaps can be used for controlled aeration of shake flasks and was successfully applied to pH buffering for cell culture

- ✓ Can be used in incubators without gas supply control as a **simple add-on**
- ✓ **Comparable pH and DO** maintained in the flasks with AerationCaps (seen in SFR data, not shown), **proven to support cell growth** similar to that in incubator-controlled gas supply

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References

[1] Möller et al. (2019) **DOI: 10.1007/s00449-019-02089-7**

[2] Möller et al. (2020) **DOI: 10.1016/j.compchemeng.2019.106693**

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