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New application for an established luminescence based sensor system - monitoring pO_2 und pH in shaker flask

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Introduction

The first steps in cell culture process development (clone screening and culture medium development) are commonly performed in simple parallel culture systems (e.g. shake flasks) without inline control of pH and pO_2. The shake flask reader system (SFR, PreSens GmbH, Regensburg) allows online monitoring of pH and oxygen without the use of sophisticated electrode integration based on chemical-optical sensors utilizing the fluorescence lifetime (pO_2) or the dual luminophor lifetime method (pH).
We have investigated the suitability of this system for k_La-determination in shake flasks and for culture control and culture medium comparison using different industrially relevant cell lines.

Methods

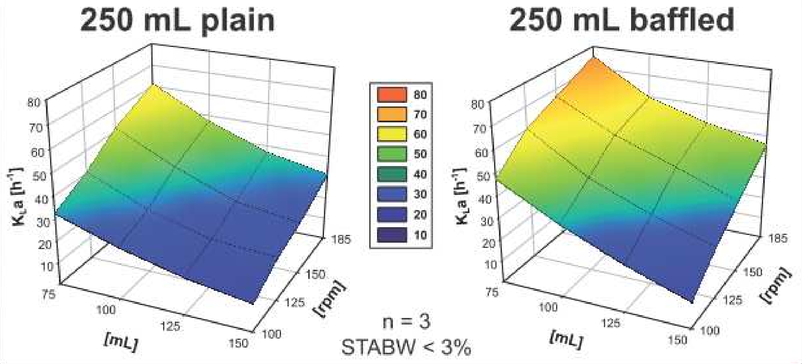
For the experiments the following cell lines were used: CHO DP12 (ATCC, CRL-12445), CHO MUC1 (University of Göteborg), MF20 (DSHB at University of Iowa), animal cell line (NN) and 14-CHO-S (Celonic AG, Basel).
All cell lines were cultivated in serum free, chemically defined, animal component free medium. Cultivations were carried out in baffled and non baffled polycarbonate Erlenmeyer flasks with vent cups (250 mL; PreSens GmbH) that contained integrated dye spots for pO_2 and pH measurement (PreSens GmbH).
Culture conditions: 80-150 mL working volume, 37°C, 5 % CO_2, 80 % humidity, shaking revolution 185 rpm, orbital movement of 2 inch.
Reference point measurements were done with standard inline pO_2 (Hamilton) and pH (Mettler Toledo) electrodes. The assembly of the reference electrodes were enabled by custom-made adapters with a Luer-Lock sampling port (DASGIP) and two air filters (Sartorius AG) for gas exchange. Analogue to the SFR, the pO_2 and pH frequency of measurement for the electrodes was set to 20 seconds. Additionally, a Blood Gas Analyzer (Diamond Diagnostics) was used to determine the pH values externally and to recalibrate the SFR and the pH electrodes at the time of the second sampling in cultivation process.
The measured values of the Hamilton pO_2 sensor were adjusted to 95 % air saturation before starting cultivation.

Results

k_La-Measurements

The SFR was used to determine the mass transfer coefficient k_La for 250 mL shake flasks under the previously described incubator conditions. The characterisation of the Erlenmeyer flasks was done for varying working volumes and different shaking revolutions.
Approach: After the degassing by aerating the deionized water with nitrogen, the increasing dissolved oxygen concentrations were recorded as fast as possible (about every 2-3 seconds). A constant measurement range of the pO_2 values (5-25 %) was used for the calculation.

250 mL plain **250 mL baffled**



*please also visit the posters:
► Scholz S. et al. - Process Development and Media Optimisation of a New Vaccine Producing Avian Cell Line
► Thüte T. *et al.* - Non-targeted metabolic comparison of production cell lines originated from different animal species
► Northoff *et al.* - Development of a new chemically defined medium for various animal cell lines

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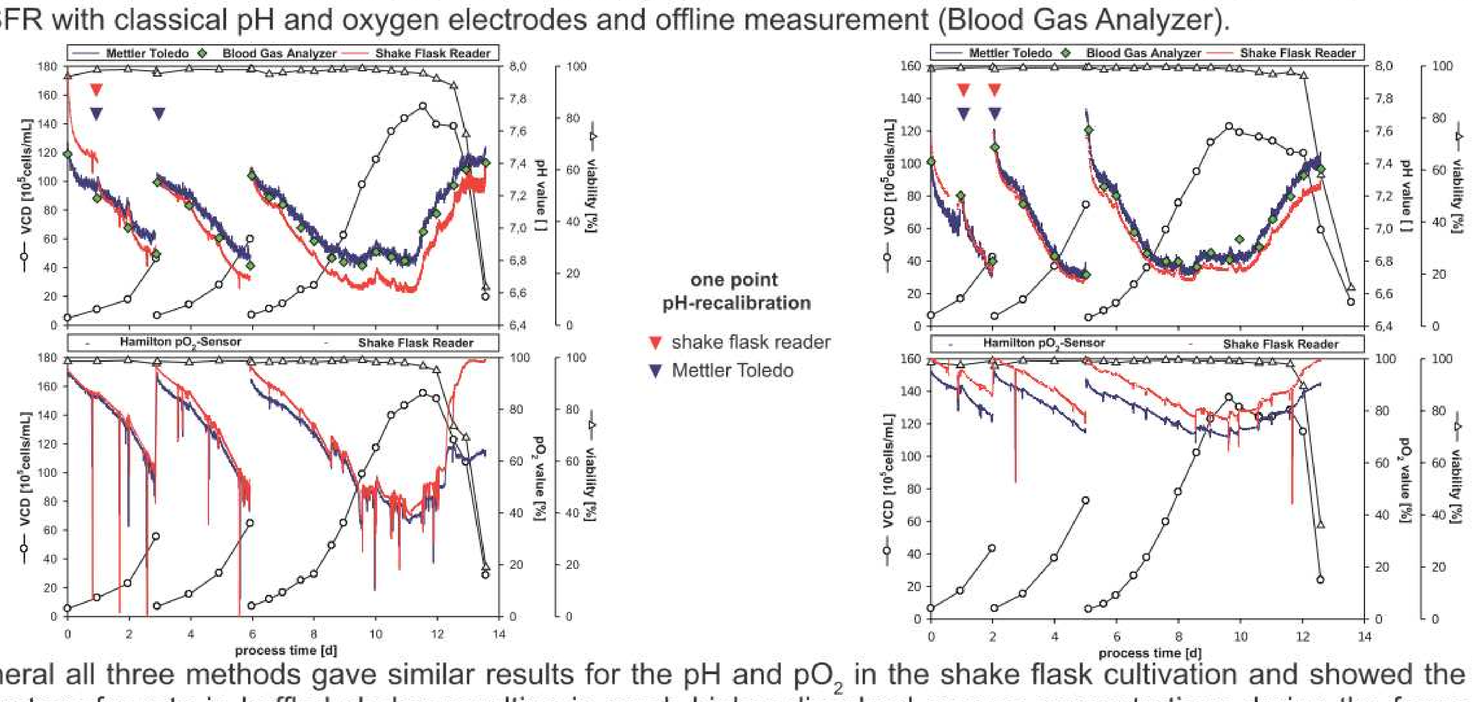
Results

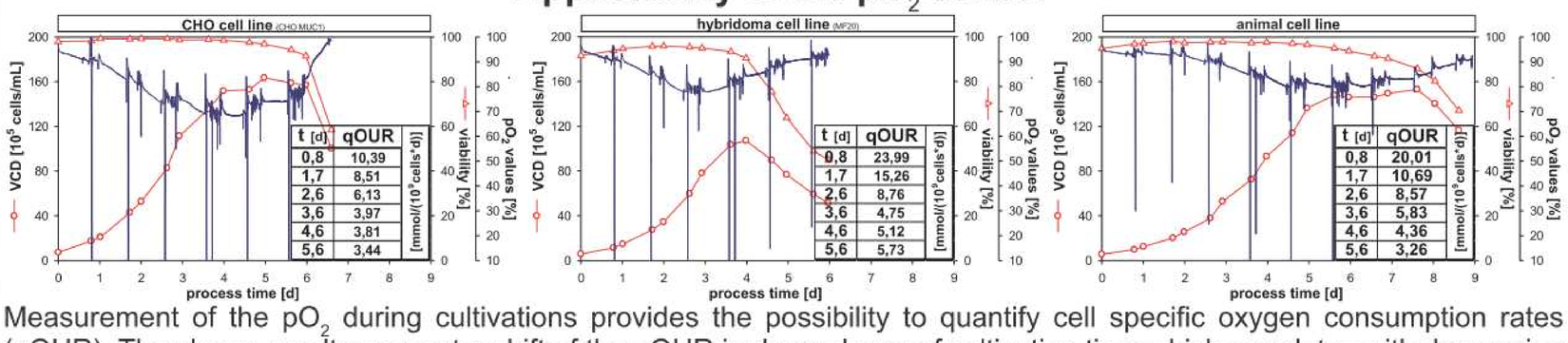
SFR dye spots versus inline pH and pO_2 electrodes

Four cultivations with non baffled (left) and baffled (right) 250 mL shaker flasks were done to compare the performance of the SFR with classical pH and oxygen electrodes and offline measurement (Blood Gas Analyzer).



In general all three methods gave similar results for the pH and pO_2 in the shake flask cultivation and showed the higher oxygen transfer rate in baffled shaker resulting in much higher dissolved oxygen concentrations during the fermentation compared to the non-baffled shake flasks (95-70% vs. 95-40%). Nevertheless some deviations have been observed. Frequently the initially measured pO_2 with the SFR is higher than the pO_2 in the incubators gas phase (95%). The reason might be a difference in atmospheric pressure between calibration of the SFR at the manufacturer and application in our lab. During cultivation an increasing difference between online pH measurement using the SFR and offline pH measurement (blood gas analyzer) occurred. This is likely caused by the very high measurement frequency (every 20s) that causes dye bleaching of the pH sensor.

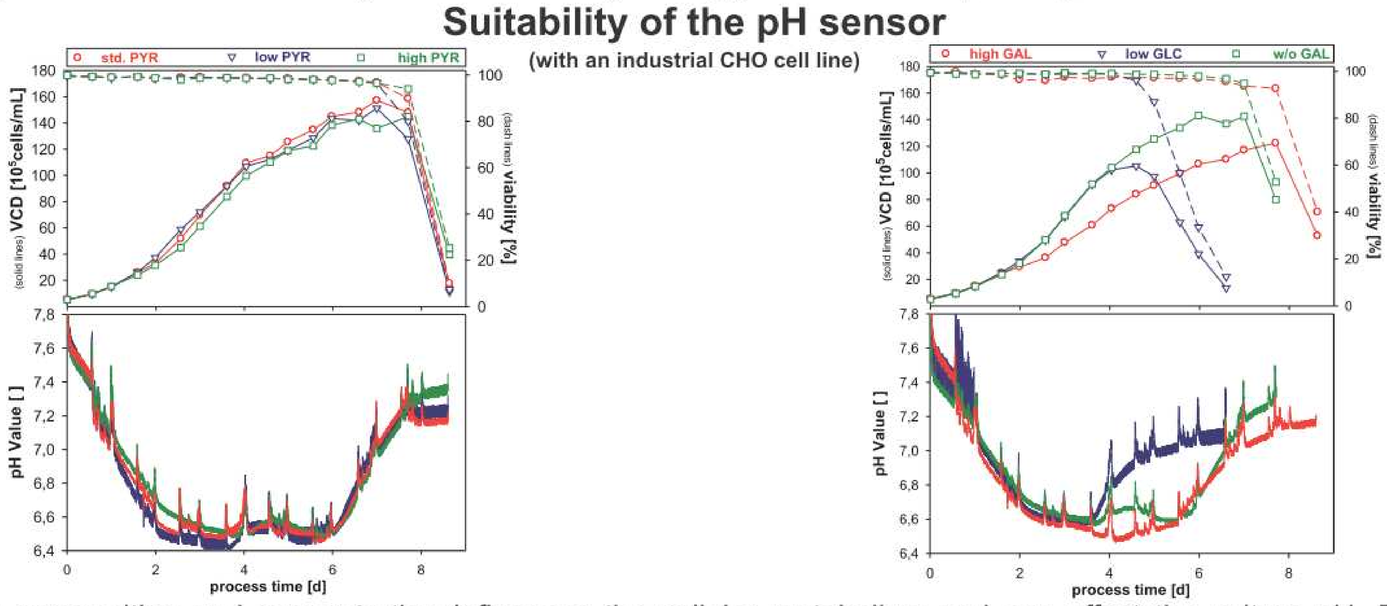
Applicability of the pO_2 sensor



Measurement of the pO_2 during cultivations provides the possibility to quantify cell specific oxygen consumption rates (qOUR). The shown results suggest a shift of the qOUR in dependency of cultivation time which correlates with decreasing substrate levels. In addition, different qOUR values for several cell lines can be observed especially during early cultivation states. Furthermore, the SFR data could verify the substantially different oxygen concentrations between shake flasks and commonly used bioreactors. This might allow estimating the oxygen demand for upscaling.

Suitability of the pH sensor

(with an industrial CHO cell line)



Substrate composition and concentration influences the cellular metabolism and can affect the culture pH. Prominent examples are the acidification caused by excessive lactate production from glucose.
The SFR allows for determination of even minor differences in pH indicating metabolic differences in the cultures as shown for pyruvate (high pyruvate concentration results in reduced glycolytic activity, less lactate accumulation and therefore higher culture pH). This can be used for medium development and selection of clones with favorite metabolic characteristics.

Conclusions

►► The SFR allows non-invasive online process monitoring of pH and pO_2 and simple determination of k_La.
►► The pO_2 values measured by the SFR provide a reliable overview of the oxygen concentration during cultivations with deviations at high dissolved oxygen concentrations. For determination of exact pO_2 values recalibration using a reference method is recommended.
►► The SFR allows for the monitoring of minor differences in pH during cultivation indicating metabolic differences between parallel cultures. The drift of the pH sensor during longer cultivations might be circumvented by reducing the measurement frequency.
►► In the shake flasks we have used in this study, variations in the position of the dye spots occurred, making the correct positioning of the shake flasks (alignment of the spots with the LEDs of the optical system) difficult, especially after sampling.
►► The pH dye spot needs several hours for moisture expansion, therefore recalibration should not be performed until spots are fully swollen.