**kLa as a predictor for probe-independent mammalian cell bioprocesses in orbitally shaken bioreactors**

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**Background**

Orbitally shaken flasks are commonly used at an early stage of bioprocess development with mammalian cells. In contrast to large-scale stirred-tank bioreactors, shaken flasks are usually operated in probe-independent bioprocesses, i.e. without strictly controlling the pH or dissolved oxygen concentration (DO). As a consequence, gas transfer issues are thought to limit the effectiveness of orbitally shaken flasks and bioreactors (OSRs). To define optimal operating conditions for probe-independent bioprocesses in OSRs, we tested the effects of the mass transfer coefficient of oxygen ($k_{La}$) on mammalian cell growth, recombinant protein production, and environmental conditions of the culture (pH, DO).

**Materials and methods**

The $k_{La}$ was measured by the dynamic method described in [1] using non-invasive O2 sensors (PreSens, Regensburg, Germany). A recombinant CHO DG44-derived cell line expressing a human IgG monoclonal antibody (CHO-IgG) [3] was cultivated in suspension as described [4]. To investigate the effects of the $k_{La}$ on cell growth, CHO-IgG cells were into 1-L cylindrical bottles with working volumes from 200 to 600 mL. The bottles were equipped with vented caps and orbitally shaken at 110 rpm in an incubator at 37°C with 5% CO2. To test the $k_{La}$ as a scale-up factor, CHO-IgG cells were inoculated at 0.3 million cells/mL in a 200-L bioreactor (7 h$^{-1}$), and the bottles were agitated at 110 rpm.

**Results**

In a 1-L OSR the $k_{La}$ decreased from 11 to 3 h$^{-1}$ as the working volume increased from 200 to 600 mL (Fig. 1a). As the working volume of the cultures increased in the 1-L OSR, the DO decreased (Fig. 1b). In all the cultures, the pH decreased with time of cultivation (Fig. 1c). At working volumes greater than 400 mL ($k_{La}$ < 7 h$^{-1}$), the maximal cell density was about 40% less than in cultures of ≤ 400 mL (Fig. 1d).

To test the $k_{La}$ as a scale-up factor for probe-independent bioprocesses, CHO-IgG were inoculated in a 200-L OSR. After overnight incubation, samples of the 100-L culture were used to inoculate satellite cultures in 1- and 5-L OSRs at volumes to give $k_{La}$ values of 7 h$^{-1}$. The cell densities were similar in the 1-, 5- and 200-L OSRs and reached 3.5 million cells/mL after 90 h (data not shown). The recombinant IgG concentrations at this time were about 150 mg/L. The pH decreased from 7.25 to 6.7 in all the cultures (data not shown), and the glucose, glutamine, lactate and glutamate profiles were similar in all the cultures.

**Conclusions**

Our results indicate that the $k_{La}$ is a good parameter to predict suitable conditions for cell cultures in probe-independent OSRs. Furthermore, our study demonstrates that cultures having different nominal scales but the same $k_{La}$ also had the same cell growth.
recombinant protein production, and culture conditions (pH and DO). The minimal $k_L a$ required to avoid pH and DO limitations in OSRs was 7 h$^{-1}$ for CHO-IgG cells. Cell cultivation in a 200-L OSR without pH or DO controllers resulted in similar cell densities, recombinant protein titers and pH values as in 1- and 5-L OSRs when the three types of OSRs were operated at the same $k_L a$. These results suggest that large-scale bioprocesses can be operated without pH or DO controllers as long as a sufficient $k_L a$ is maintained through appropriate cultivation conditions (e.g. working volume, agitation rate, geometry of the vessel).

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