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k_La 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 O_2 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% CO_2. 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 OSR (Kühner AG, Birsfelden, Switzerland) with a working volume of 100 L and agitated at 57 rpm. Air containing 5% CO_2 was flushed into the OSR at 1 L min^{-1}. After overnight cultivation, samples were withdrawn from the 100-L culture and used to inoculate satellite cultures in 1- and 5-L bottles with vented caps. The volume of the cultures in bottles was adjusted to obtain the same k_La as the one in the 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,

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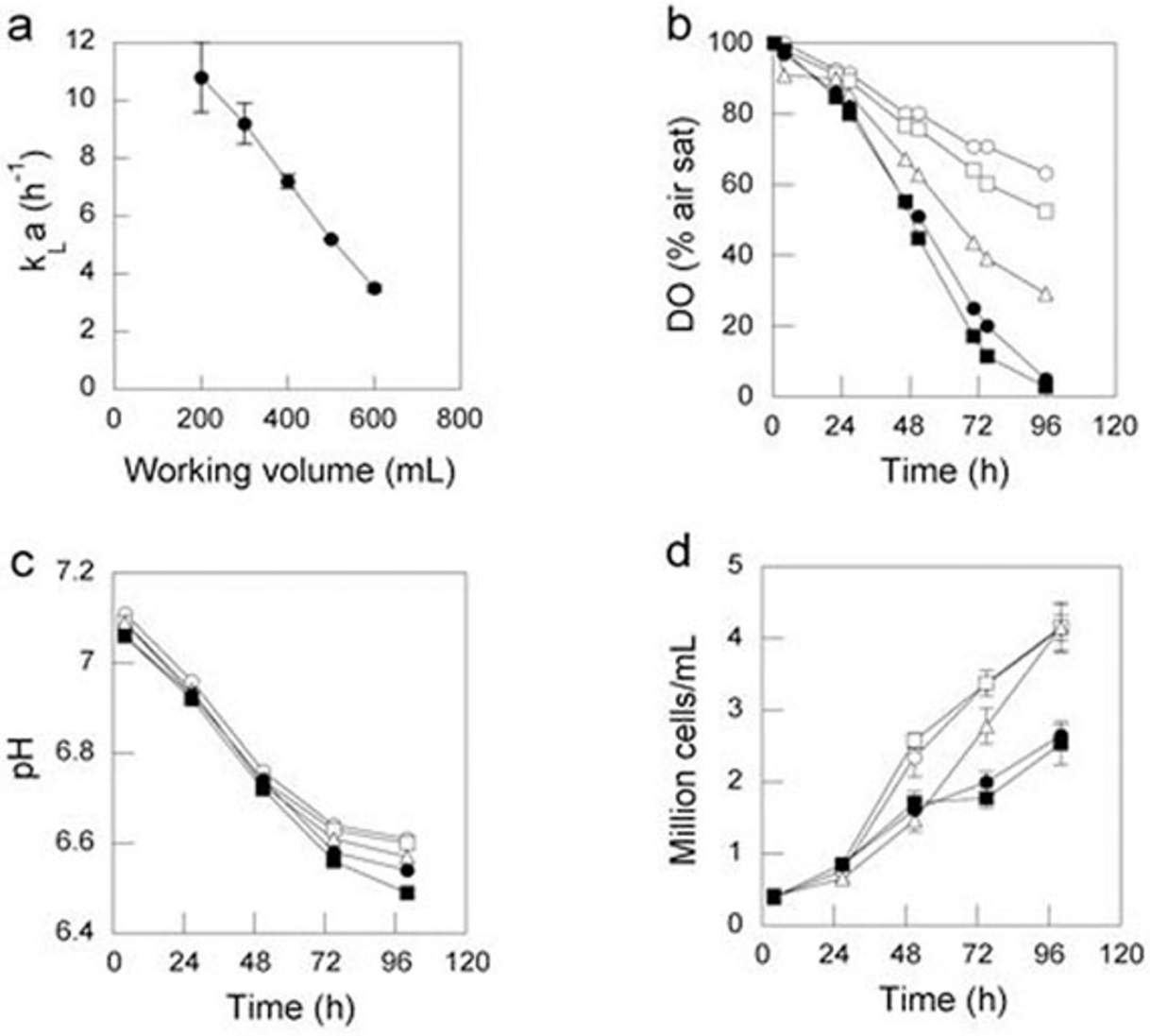

Figure 1 Effects of the $k_L a$ on CHO-IgG cell cultures. The $k_L a$ was measured in 1-L OSR with working volumes from 200 to 600 mL (a). The CHO-IgG cells were cultivated in 1-L OSR in 200 (□), 300 (□), 400 (Δ), 500 (●) and 600 mL (■). The DO (b), pH (c) and viable cell density (d) were measured at the times indicated. The shaking diameter was 5 cm.

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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