Where Does the Oxygen Go?

k_La Measurement in Bioreactors

Small plastic tubes are available as single-use bioreactors for cell cultivation as a cost effective alternative to larger scale cultivation systems. However, the characteristics of the tubes with regard to oxygen transfer have hardly been investigated. Using a specially adapted shake flask reader (SFR) and chemical optical sensors with rapid response time, the volumetric oxygen mass transfer coefficient (k_La) for tube systems was determined. The characteristics of these cultivation vessels were investigated more closely, which represents an important step towards defined screening processes by monitoring oxygen in bioreactors.

Disposable bioreactors are used for cell cultivation at milliliter scale in orbitally shaken incubators. Thus, development of optimized cell culture media and identification of specific cell lines can be carried out quickly and inexpensively [1]. Currently, there is very little information in the literature about oxygen transfer in bioreactors. In this regard, Zhang [2] studied the characteristics of bioreactors and listed k_La values up to 29 h^{-1}. The volumetric oxygen mass transfers coefficient (k_La) indicates the ability of oxygen transfer from the gas phase to the liquid phase [3].

The Shake Flask Reader

The Presens SFR is commercially available since 2009. It allows the measurement of dissolved oxygen (DO) in shake flasks with integrated disposable sensors, which are read out by a special reader unit mounted inside the shaker. The recorded data is transferred to a PC via wireless Bluetooth connection. This system was further developed and extended by the company so it can be used with tube bioreactors. The extensions comprise special adapters to hold the tubes and read DO values by means of the integrated disposable sensor spots (fig. 1). DO measurement in these reactors can be applied for monitoring screening processes with regard to oxygen consumption of the cells. Furthermore, it is now possible to characterize tube bioreactors more precisely by measuring the k_La values.

Optical Oxygen Measurement

DO measurements are conducted with chemical optical sensors attached to the bottom of the bioreactors (fig. 1). A major advantage compared to conventional oxygen probes (e. g. Clark-type electrodes) is the miniaturized size of the sensor spots. The integration of larger conventional oxygen probes would change the flow behavior of the liquid inside the reactors. Moreover, these tube bioreactors are too small for conventional probes, and due to the orbital shaking movement the liquid is not always in contact with the probe. Additionally, the sensor spots respond quickly within 10 – 30 s, which outranges conventional oxygen sensors. In 2002 such spots were already being used to determine k_La in shake flasks, and reliable values of up to 150 h^{-1} could be measured.

Medium, Methods & Equipment

The oxygen transfer rate (OTR) is measured at 25°C in a coalescence reduced aqueous solution (0.2 M Na_2SO_4) using the dynamic sulfite method [4]. When Co^{2+} is present, sulfite reacts to sulfate consuming oxygen (chemical oxidation) until the DO level reaches the 0 % value (oxygen depletion). Starting from that point, the DO amount is recorded using defined parameters (filling volume, frequency, amplitude) at intervals of 10 s until 95 % saturation is regained. k_La values are calculated from the measured DO by linearization of the response in a range from 20 % to 80 % of saturation concentration (oxygen saturation being realized by diffusion from ambient air using vented caps). The k_La in tube bioreactors was measured with varying volumes (10 – 30 ml) and shaking frequencies (150 – 300 rpm). Furthermore, amplitudes of 25 and 50 mm, which are both common to laboratory shakers, were investigated. A statistical design of experiment (DoE) was used to determine the number of experiments and evaluate the data.

Results

k_La values in a range of 0.5 h-1 ± 30 % up to 21 h^{-1} ± 10 % were determined in the bioreactors (tab. 1). In single-use shake flasks, k_La values of up to 104 h^{-1} were determined. After evaluation of data, the following correlation of filling volume (FS), frequency (FR), and amplitude (AM) in tube bioreactors was identified using statistics software Visual XSel 11.0:

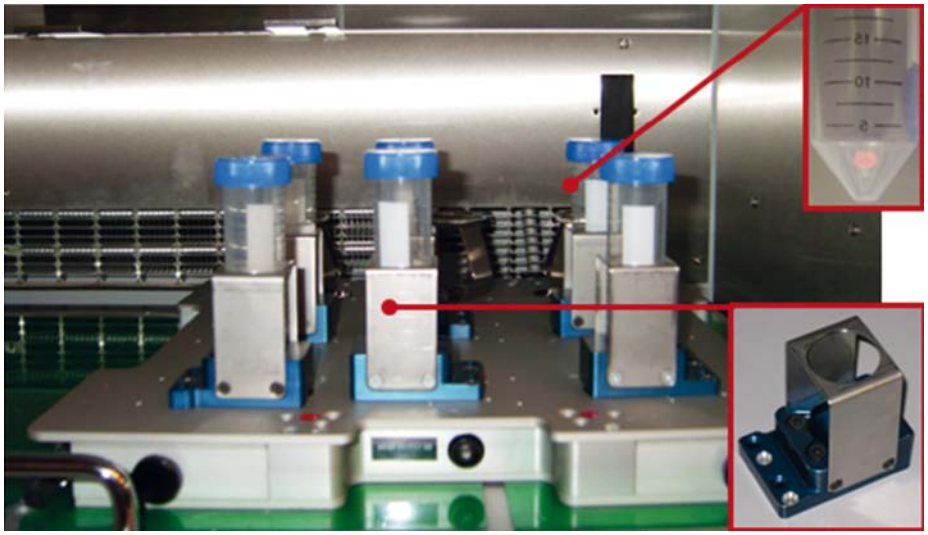

Fig. 1: Bioreactors on the SFR; upper inset: integrated sensor spot for DO measurement; lower inset: holder designed for tube bioreactors

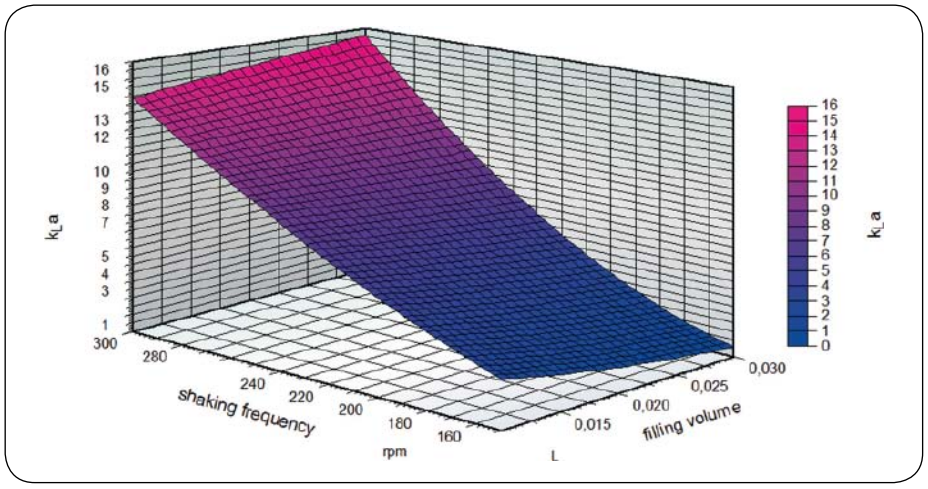

Fig. 2: k_La values at a set amplitude of 50 mm with varying filling volumes and frequencies. With increasing frequency the k_La value is no longer influenced by the filling volume; this model does not show that k_La values increase with larger filling volume and higher rotation speed.

$k_La = (1.196007 + 4.089 \times 10^{-3} \times FR - 80{,}2998 \times FS\ 0.04206 \times AM + 0.166267 \times FR \times FS + 2.53 \times 10^{-4} \times FR \times AM + 0.633247 \times FS \times AM)^2$

This correlation can be displayed in a three-dimensional surface plot (fig. 2). The highest k_La values were achieved at an amplitude of 50 mm. Interestingly, experiments showed that high filling volumes with an amplitude setting of 50 mm and a high shaking frequency (> 250 rpm) resulted in high k_La values in the bioreactors. This behavior may be caused by an increased liquid-to-air-surface area and a larger area where the liquid is in contact with the vessel wall, which is supported by the cylindrical shape of the tubes. The liquid rotates as a thin film along the vessel wall, which may increase gas mass tranfer into the liquid (fig. 3). Such an effect cannot be observed in shake flasks as fluid rotation is only possible in the lower part of the bioreactor due to the conical shape of the vessels [5]. These effects cannot be predicted by the statistical model at present, which makes further testing mandatory. With a k_La value of 21 h^{-1}, Chinese hamster ovary (CHO) cells with a specific oxygen consumption rate of 9 x 10^{-12} g (cell h)$^{-1}$ can be grown to achieve cell densities of more than 56 x 10^6 cells ml^{-1} under non-limiting conditions.

Bioreactor	k_La	Frequency [min^{-1}]	Filling Volume [ml]	Amplitude [mm]
Shake flask				
Min	0.7	80	200	25
Max	104	210	50	50
Tube reactor				
Min	0.5	150	30	25
Max	21	275	30	50

Tab. 1: Minimum and maximum k_La values in shake flasks and tube bioreactors.

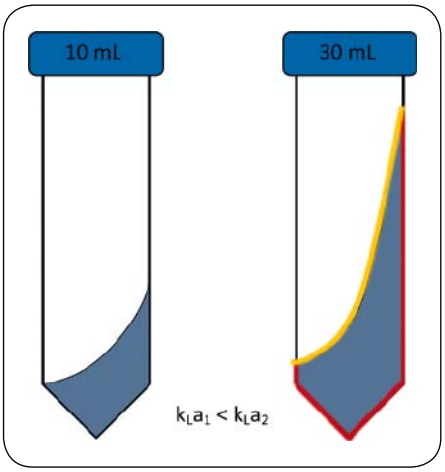

Fig. 3: Schematic illustration of fluid behavior at higher shaking frequency, 50 mm amplitude, and different filling volumes (left: 10 ml, right 30 ml); the specific exchange area (yellow line) and the contact area (red) increase with larger filling volume, which enhances oxygen transfer.

Conclusion

DO measurements can be conducted faster and more efficiently with the shake flask reader than with conventional invasive sensor probes. The relatively free choice of measurement frequencies, and the fast response time of the disposable sensors enable a stable DO measurement. Culture conditions can be monitored and adjusted as necessary with the miniaturized sensor spots even in small, simple bioreactors such as the applied tube reactors. Using the SFR, these „black-box" bioreactors are more accessible with regard to oxygen transfer investigations. This is an important step towards defined screening processes. A statistical correlation of motion parameters and the specific oxygen mass transfer coefficient k_La in bioreactors was determined using this system (tab. 1).

References available at the editorial office.

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