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Life Sciences

Determination of k_La values on the Cydem VT Automated Clone Screening System

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An important process parameter of bioreactors is the oxygen transfer rate (OTR), describing how fast oxygen is transferred from the gaseous phase (supply gassing) into the aqueous phase (media). The OTR can be calculated using the volumetric mass transfer coefficient (or k_La) and the difference between the actual dissolved oxygen concentration (DO) and the dissolved oxygen saturation concentration (DO*):

$$OTR=k_La*(DO*-DO)$$

During aerobic cultivations – for example with mammalian cell culture – it is crucial to ensure sufficient oxygen supply to the organisms. Unfortunately, oxygen solubility is low in commonly used cultivation media. Therefore, bioreactor design elements like geometry, volume-to-surface ratio, stirrer type and gassing system, as well as process parameters like agitation rate, filling volume and aeration rate, need to be optimized to achieve the required OTR.

The k_La comprises the volumetric interfacial gas-liquid surface area (a) and the mass transfer coefficient (k_L) – which both are extremely difficult to determine experimentally. So, for the characterization of bioreactors, the k_La is determined as a single coefficient. There are several methods for k_La determination, some of them based on saturation curves like the gassing-out method from Bauer et al., published by DECHEMA.[1] The described method was used to determine the k_La of the bioreactor module of the Cydem VT Automated Clone Screening System. Additional methods, both experimental and numerical, are detailed in Seidel et al., 2021.[2]

Methods

DoE Approach

A central composite design (CCD) was generated using the DoE functionality in JMP3. This design is used to fit a second order polynomial model with the provided covariates, and this is preferred over a linear model due to expected interaction between the covariates and curvature in the response. Additionally, a CCD design allows one to find optimal conditions for the response in the design space.

For this use case the k_La value is the response, while rotational velocity and filling volume are the covariates. A CCD with axial values on the faces of the design space were used, two additional center points were added, and the number of replicates was two.

In the gassing out method, the measured k_La values correspond to a calculated regression coefficient, which have their own errors. Some of the calculated k_La values only used the minimum number of samples specified in method from Bauer et al., while others used up to triple that amount. Due to this discrepancy, the calculated k_La values have different standard errors. To accommodate measurement errors when fitting the second order polynomial model, weighted least squares (WLS) can be used over ordinary least squares (OLS), where the weights in WLS are inversely proportional to the standard error of the calculated k_La coefficients.

DO-saturation curve data was generated at different filling volumes and shaking frequencies, spanning the operating conditions of the bioreactor module of the Cydem VT system. The wells were filled with phosphate buffer saline (PBS) solution, inserted into the bioreactor module and incubated at 37 °C. According to the described measurement procedure,[1] the first step was to eliminate all oxygen from the system. This was achieved by gassing with nitrogen while continuously measuring the dissolved oxygen via the immobilized optode on the plate bottom. Once all oxygen was removed, the orbital shaker was paused and the gas in the headspace was exchanged three times with air. Afterwards, the shaker was started again under continuous air gassing until the DO signal saturated again (**Figure 1**).

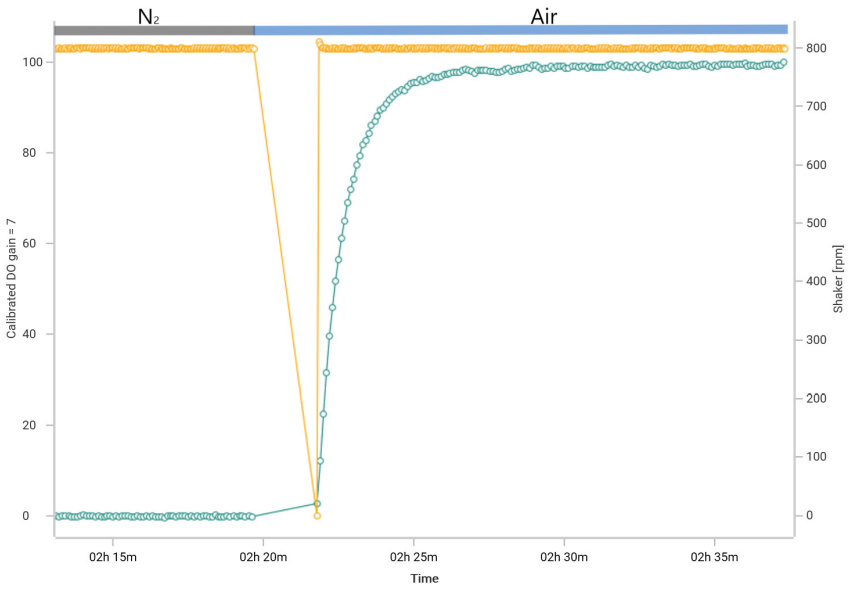

Figure 1. Dissolved oxygen saturation curve (green) during a k_La determination experiment. The shaker (yellow) was stopped briefly after air gassing was started to flush the headspace.

Saturation curves were collected at 600, 700 and 800 rpm at filling volumes of 3, 4.5 and 6 mL. All runs were performed in triplicate, except for the 700 rpm, 4.5 mL condition with six replicates.

Results

The generated saturation curves were analyzed to determine the k_La at the defined experimental conditions. These data were used to interpolate values across the whole space of supported filling volumes and shaking frequencies during a clone screening experiment (**Figure 2**).

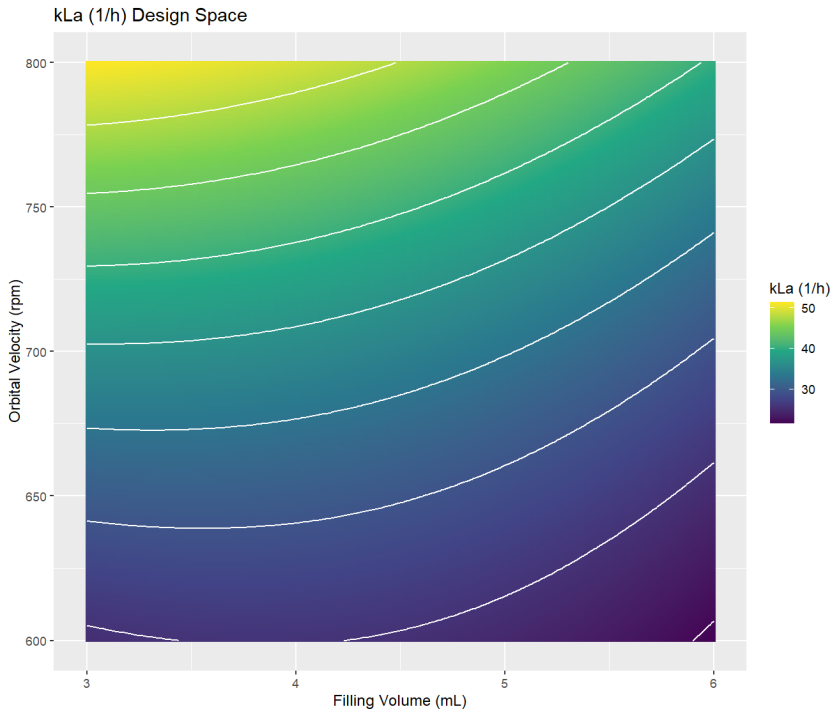

Figure 2. Dependency of k_La on shaking frequency and filling volume.

As expected, the k_La increased with increasing shaking frequency and decreasing filling volume – due to higher surface-to-volume ratios (**Figure 3**).

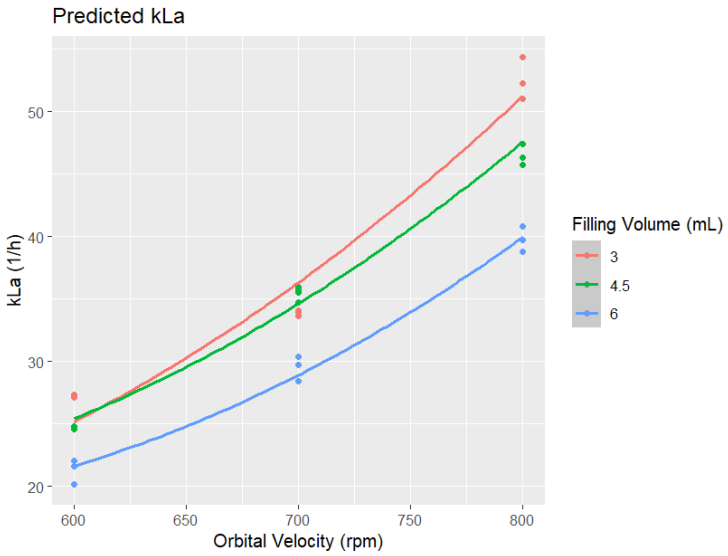

Figure 3. Prediction of k_La overlayed with experimental measurements.

Keeping k_La values constant during scale-up is a key scaling factor for mammalian cultivations. The predicted k_La values correspond well with previously reported values for other mammalian cultivation systems. For shake flasks, microtiter plates and TubeSpin reactors, k_La values were calculated to be in the range of 30 to 40 h-1,[4,5] which are at the same order of magnitude as the predicted values for the Cydem VT system.

Literature

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2025-GBL-EN-107445-V1