TECHNICAL NOTE

S.NEST™ | Mass transfer coefficient (K_La) of the S.NEST microbioreactor

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Introduction

Engineered mammalian cells and microbes are often used to produce protein products in large-scale bioreactor systems in the biopharmaceutical and biotechnology industries. For aerobic cultivation, dissolved oxygen (DO) is a fundamental substrate for cell or microbe growth, maintenance and production. To find optimal culture conditions for bioreactors, the oxygen mass transfer coefficient (K_La) is a critical reference for bioprocess scale-up in the process development stage. The K_La is the parameter that controls the rate of oxygen transfer from the gas phase into the liquid phase and can be affected by multiple factors of a bioreactor, such as mixing rate, airflow rate, gas bubble size, different liquid or medium, etc. [1]

CYTENA's S.NEST microbioreactor and the S.NEST lid allow the suction and expulsion of air into and out of each well in a standard cell culture plate, enabling continuous reciprocal mixing at different mixing periods in each well (**Figure 1A**). Therefore, the S.NEST microbioreactor introduces suspension culture and late-stage conditions to the early-stage cell line development (CLD) pipeline, providing more growing space and oxygen than static cultures in 96-well and 24-well plate cultures. We have shown that our mixing culture system of microplates can significantly increase the oxygen transfer into the aqueous medium, providing a better culture environment for aerobic cultivation compared to static culture. [2] Accordingly, our mixing culture improves the cell growth rate with both adherent cell line HEK293 and suspension cell line CHO-S. [3,4]

A remarkable feature of the S.NEST microbioreactor is the real-time monitoring of DO and pH values during the entire cell culturing process using the optical DO and pH sensors attached to the bottom of each well (**Figure 1B**). Here, we demonstrated the K_La in the S.NEST microbioreactor with different working volumes and mixing rates using the built in DO sensors of the S.NEST plate.

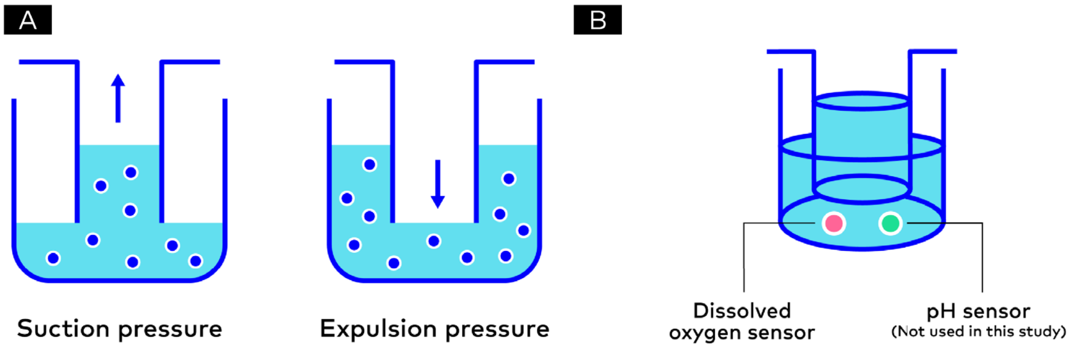

Figure 1. ***(A)*** *The principle of continuous reciprocating mixing provided by the S.NEST system.* ***(B)*** *Optical sensors are attached to the bottom of each S.NEST plate well to monitor the DO and pH value.*

Materials and methods

In this study, all the experiments were performed in our S.NEST microbioreactor with 1,000 μL or 1,400 μL DPBS (21-031-CM, Corning) at 37°C. The test mixing rates include 50, 30, 10, 5 and 2 seconds per cycle. We used the gassing out method to determine the K_La. This method contains 3 phases: the calibration phase, the oxygen-depleting phase and the oxygen-dissolving phase (**Figure 2**). In the calibration phase, the DPBS was added to the S.NEST 24-well plate, lidded with the S.NEST lid and mixed for 1 hour with 10 seconds/cycle of continuous mixing rate in the S.NEST to ensure the DO saturation. The DO level was then calibrated to 100%. The next phase was the oxygen-depleting phase, in which the N_2 was supplied to the S.NEST to deplete the DO until the DO concentration reached the lowest level. The last phase was the oxygen-dissolving phase, which replaced the N_2 supply with the air. The DO levels were monitored in real time through all three phases every 60 seconds. The time course used to determine the K_La is shown in **Figure 2** (red box) and the K_La value can be determined by the equation as follows:

$$\frac{dc_L}{dt} = K_L a(C_L^* - C_L)$$

Where:

K_L = mass transfer coefficient (cm/h)

a = gas-liquid exchange area per unit of liquid volume (cm²/cm³)

C^*_L = the saturated DO concentration in the liquid (mmol/L)

C_L = local DO concentration in the liquid (mmol/L)

Assuming the K_La and C^*_L are constant during the testing process, integration of the equation becomes the following equation:

$$ln\left(\frac{C_L^* - C_{L1}}{C_L^* - C_{L2}}\right) = K_L a(t_2 - t_1)$$

Where:

C_{L1} and C_{L2} = the local DO concentrations in the liquid at t_1 and t_2

Therefore, the slope of this equation can be regarded as the K_La.

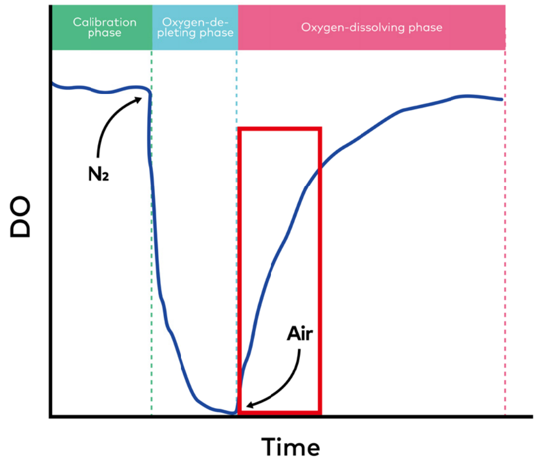

Figure 2. *The time course of DO in the K_La determination process. The red box indicates the time course of DO used to determine the K_La.*

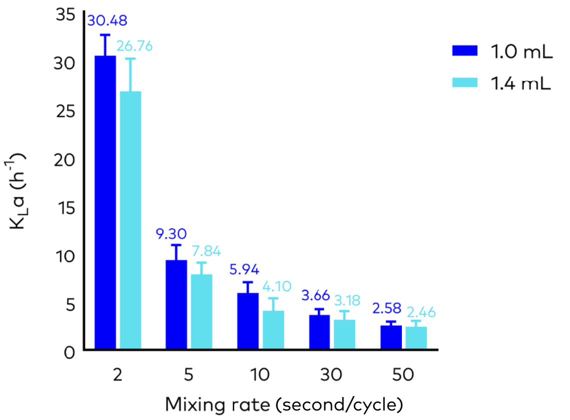

Figure 3. *The K_La values of the S.NEST with 1.0 mL and 1.4 mL of DPBS under different mixing rates. The error bars correspond to standard deviation.*

Results and discussion

Mixing rate

Mixing rate was the critical factor affecting the K_La of the S.NEST. In this study, we tested five mixing rates ranging from 2 to 50 seconds/cycle. The K_La increased with the mixing rate (**Figure 3**). The maximum K_La reached up to 30.48 h^{-1} with 1.0 mL DPBS at a mixing rate of 2 seconds/cycle.

Based on the K_La of this study, we analyzed the correlation between the K_La and the mixing rate (**Figure 4**). The K_La was proportional to the mixing rate^{-1}. Therefore, these curves could be used as a guide to select desired mixing rate. As referenced, **Figure 5** shows the K_La value of the Sartorius Ambr® 15 Cell Culture system (**Figure 5A**) and a 5L bioreactor (**Figure 5B**) under different conditions [5,6].

Working volume

The working volume was another critical factor affecting the K_La of the S.NEST. Increasing the working volume had a negative impact on the K_La. In this study, the K_La with 1.0 mL working volume were all about 15% higher than the K_La with 1.4 mL working volume at the same mixing rate (**Figure 3**).

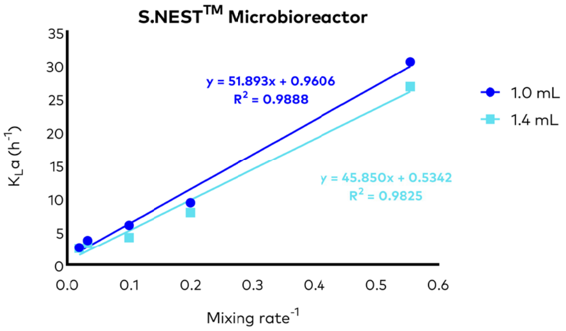

Figure 4. *Correlation between the K_La and the mixing rates in the S.NEST microbioreactor.*

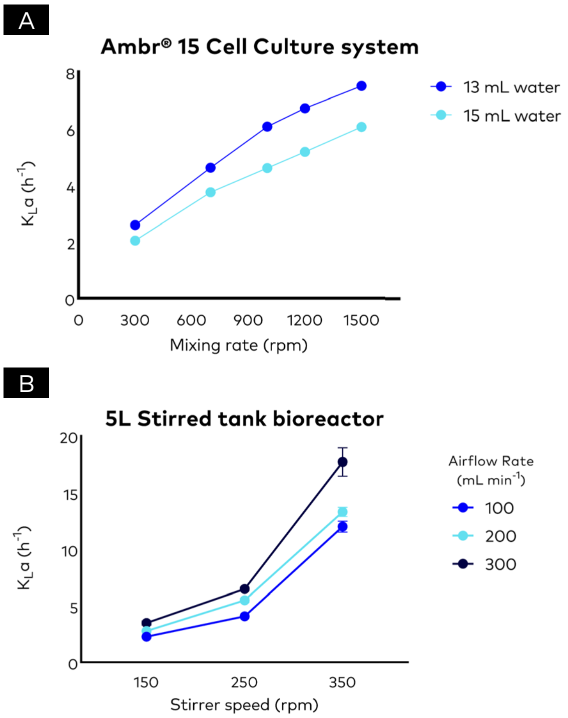

Figure 5. *The K_La values of **(A)** Ambr 15 Cell Culture system and **(B)** a 5L stirred tank bioreactor under indicated conditions.*

Conclusion

In this technical note, we demonstrated that the K_La value is adjustable in the S.NEST microbioreactor. Adjusting the liquid volume or the mixing rate can result in a different K_La value and therefore can be used to determine the optimal culture conditions for aerobic cultivation. The built-in DO sensors in the S.NEST culture plate can also enable users to manually determine the K_La value under desired conditions for further process transfer and scale up.

References

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Edited version: September 2023 | CBS_PUB_SNEST_Tech-Note-03_Digital