

S.NEST | Mass transfer coefficient ($K_L a$) 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 ($K_L a$) is a critical reference for bioprocess scale-up in the process development stage. The $K_L a$ 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.

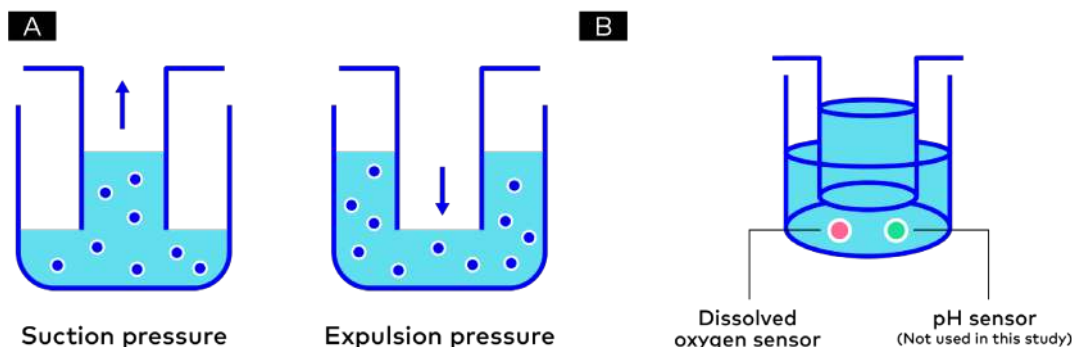


Figure 1. (A) The principle of continuous reciprocating mixing provided by the S.NEST system. (B) Optical sensors are attached to the bottom of each S.NEST plate well to monitor the DO and pH value.

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:

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

Where:

K_L = mass transfer coefficient (cm/h)

a = gas-liquid exchange area per unit of liquid volume (cm^2/cm^3)

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:

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

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 .

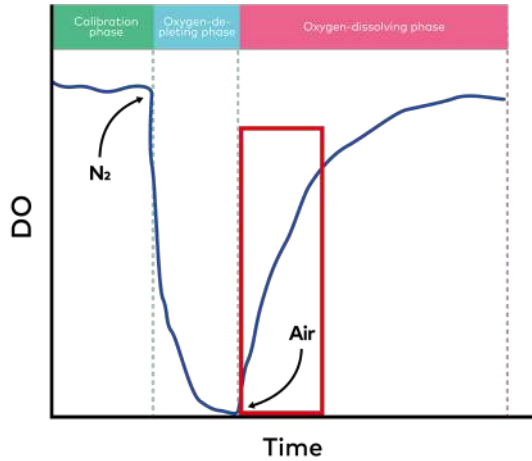


Figure 2. The time course of DO in the K_La determination process. The red box indicates the time course of DO used to determine the K_La .

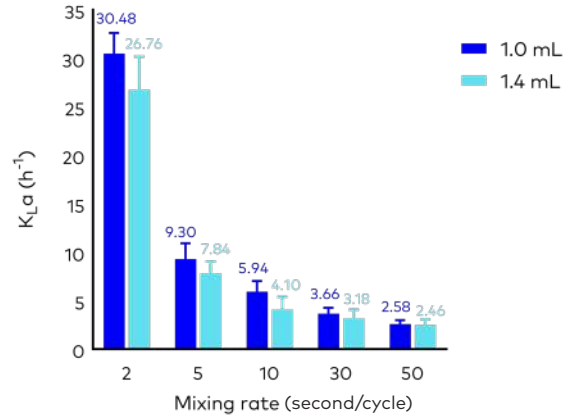


Figure 3. The K_La values of the S.NEST with 1.0 mL and 1.4 mL of DPBS under different mixing rates. The error bars correspond to standard deviation.

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**).

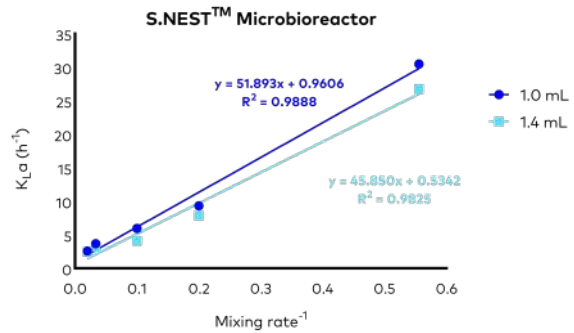


Figure 4. Correlation between the K_La and the mixing rates in the S.NEST microbioreactor.

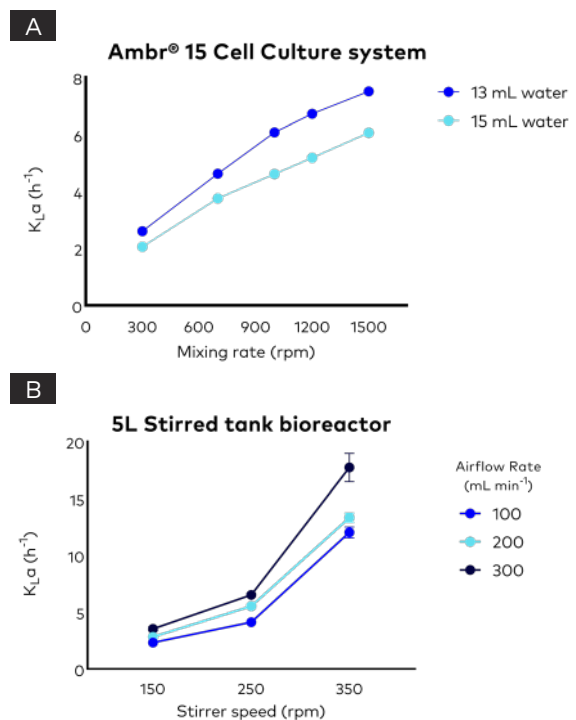


Figure 5. The K_La values of (A) Ambr 15 Cell Culture system and (B) a 5L stirred tank bioreactor under indicated conditions.

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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