

Oxygen transfer in shake flask cultures

Oxygen transfer is a key factor in shake flask cultures, yet it's often unmeasured. Insufficient oxygen can lead to slower growth, metabolic shifts, and scale-up challenges. This white paper outlines how to characterize kLa and improve oxygen availability in your experiments.

Introduction

Shake flasks are widely used for microbial and cell culture experiments due to their simplicity, cost-effectiveness, and ease of handling. However, one of their **major limitations** is **oxygen transfer**. In aerobic cultures, the transfer of oxygen from the gas phase to the liquid phase is not always sufficient to meet cellular demands. The oxygen transfer rate (OTR) in shake flasks is influenced by factors such as shaking speed, flask geometry, liquid volume, temperature and medium composition. It is therefore crucial to systematically characterize oxygen transfer at the exact conditions of your cultivation, to get an accurate estimate.

When oxygen availability is not properly assessed, cultures can become oxygen-limited, leading to:

- **Reduced growth rates** – Cells slow down or enter stationary phase too early.
- **Byproduct formation** – Anaerobic metabolism can lead to unwanted side products.
- **Irreproducible results** – Variations in oxygen availability between experiments lead to inconsistencies.
- **Scale-up failures** – Shake flask conditions do not match bioreactor environments, causing unexpected challenges during process transfer.

The ShakeReactor

Turn flasks into bioreactors with the ShakeReactor. This wireless, battery-powered bioreactor takes up the same amount of space as a standard flask and can be set up in just 10 minutes. The ShakeReactor attaches onto 500 mL and 1000 mL Erlenmeyer flasks and works with standard incubators and shakers.

The reactor contains 3 high precision pumps that can be freely programmed for feeding, induction, and pH control.

Online-measurements of pH, dissolved oxygen (DO), ambient temperature and humidity are collected wirelessly.



Increasing shaking speed or reducing liquid volume is often used to improve oxygenation, but without measuring the effect **these adjustments remain guesswork**.

This white paper provides a **practical guide** on how to **characterize oxygen transfer in shake flasks**, focusing on:

- Understanding mass transfer coefficients and their role in characterizing oxygenation
- Methods for measuring and predicting kLa in shake flasks
- Practical recommendations for optimizing oxygen availability in shake flask cultures

Describing oxygen transfer

Shake flask cultivations are typically carried out in vessels filled with **5–30% liquid**, sealed with a filter closure to allow gas exchange while preventing contamination. In these systems, oxygen is distributed across **three distinct interconnected zones**; the cultivation medium, the headspace of the flask and outside the flask. Transfer of oxygen to the cultivation medium depends on three processes:

1. Transfer from the atmosphere into the headspace, through the filter closure.
2. Transfer into the cultivation medium from the headspace, across the gas-liquid interface.
3. Consumption of oxygen by cells in the cultivation medium.

To ensure adequate oxygen availability, it is essential to **characterize and quantify the two transfer steps**. Each can be modelled as the **conductivity** of the interface to oxygen, multiplied by the **oxygen concentration difference** across the interface.

1. Transfer across the filter closure:

$$OTR_{filter} = k_W (C_o - C_g)$$

2. Transfer across the gas-liquid interface

$$OTR_{gas/liquid} = k_L a (C_{eq} - C_l)$$

Where the conductivity to oxygen transfer over each interface is summarized in the empirically determined mass transfer coefficients, k_W and $k_L a$. C_o , C_g , and C_l are the oxygen

concentration in atmosphere, headspace and cultivation media respectively. C_{eq} is the equilibrium oxygen concentration in the liquid at a given gas phase concentration, it can be related to C_g using **Henry's law**— $C_{eq} = k_H C_g$ — k_H is well known for oxygen in water, and mainly depends on temperature and pressure, functions or tabulated values can therefore be found in literature e.g. the NIST chemistry Webbook.

k_W is mainly dependent on the type of filter closure, area and temperature, and is largely independent of other experimental conditions. K_a however, is **highly dependent on the experimental conditions**, it depends on flask geometry, flask material, shaking orbit diameter, shaking speed, fill level, temperature and over all media composition. Empirical determination for your laboratories most common settings is therefore key in ensuring reproducible oxygenation between experiments.

Ensuring sufficient oxygen transfer

When k_W and K_a is known they can be used to **estimate the maximum oxygen transfer rate** (OTR_{max}) into a cultivation using the following formula:

$$OTR_{max} = k_L a k_H \frac{C_o * k_W}{k_L a * k_H * V_l + k_W}$$

Where V_l is the volume of liquid in the flask. Based on measurements of K_a at different fill and shaking speeds, the maximum oxygen transfer can then be predicted, such as in figure 1.

The last step in ensuring sufficient oxygen transfer is making sure that OTR_{max} for your experimental conditions is larger than the **oxygen uptake rate (OUR)** of the organism. OUR can be estimated from table values of specific oxygen consumption, q_{O_2} , multiplied by the expected biomass, X :

$$OUR = q_{O_2} X$$

Specific oxygen consumption can be found in literature for most species and are approximately transferable from clone to clone. A **good rule of thumb** is to assure that OTR_{max} is 20-30% larger than the maximum expected OUR.

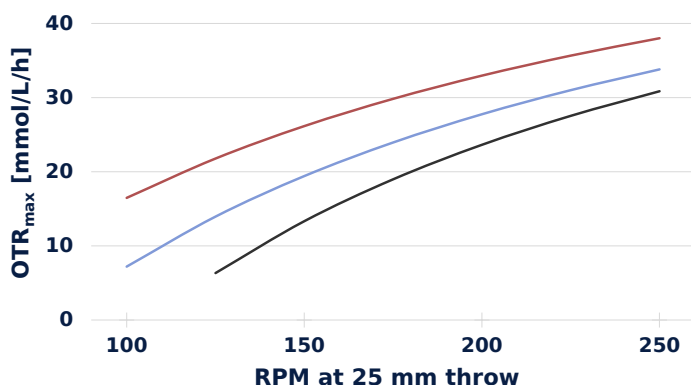


Figure 1 — OTR_{max} as a function of RPM for 100 mL —, 200 mL — and 300 mL — fill in a 1 L baffled Corning polycarbonate flask closed with a filter with k_W of $2.67 \times 10^{-7} \text{ m}^3/\text{s}$

Measuring K_a in Shake Flasks

Historically, measuring the K_a in shake flasks required custom-built equipment, limiting its accessibility. However, advances in off-the-shelf dissolved oxygen (DO) measurement technology have made K_a determination more accessible. Both optical and electrochemical DO sensors are now available for shake flasks, allowing researchers to characterize oxygen transfer.

A common method for measuring K_a is the **dynamic nitrogen outgassing method**. This method requires a DO sensor for real-time oxygen monitoring, a pressurized nitrogen source to displace oxygen from the medium, a source of atmospheric air (e.g., a manual pump or gas line) to flush the headspace and two sampling ports.

The **ShakeReactor** provides DO monitoring and has two Luer sampling ports for nitrogen and air connections, making it an efficient system for K_a characterization.

K_a , measurements should be performed at **varied fill levels and shaking speeds (RPM)** under typical incubation conditions. K_a generally follows a linear or slightly exponential trend with fill volume and RPM. Three measurement points per variable are usually sufficient to establish a reliable correlation.

The following protocol details **how to measure K_a independently of k_W using the ShakeReactor**, allowing the obtained values to be applied to identical flasks used without the device.

Protocol for K_a

1. Setup your flask at the conditions to test
2. Setup the flask in a system with online monitoring of dissolved oxygen, such as the ShakeReactor
3. Adjust sampling ports such that one sits in the liquid and one sits in the headspace
4. Record a 100% oxygen saturation baseline while shaking
5. Connect nitrogen and purge the liquid through the sampling port while shaking until a stable 0% DO reading is achieved.
6. Stop shaking and flush headspace through the other sampling port with a volume of atmospheric air at least three times larger than the headspace volume.
7. Restart shaking and record the rebound in dissolved oxygen as a function of time.
8. Dissolved oxygen in saturation percentage over time for the rebound from 10% to 90% is fitted to the equation:

$$C_{l,\%}(t) = 1 - e^{-k_L a t}$$

1. Or its linearized form using linear regression:

$$\ln(1 - C_{l,\%}) = -k_L a t$$

Measuring k_w for Filter Closures

Determining k_w , the gas exchange coefficient for filter closures, is more complex than measuring k_a , as the filter sits on the flask mouth. Accurate measurement requires a **shake flask with a second neck** to accommodate a DO sensor while maintaining a sealed system.

For **1 L flasks or smaller** with **filter paper-based closures**, typical k_w values range between **$1\text{--}10 \times 10^{-7} \text{ m}^3/\text{s}$** . Contact atSpiro for assistance in determining values for specific filter configurations. The ShakeReactor and Corning Erlenmeyer polycarbonate flasks with vented cap have $k_w \approx 2.5 \times 10^{-7} \text{ m}^3/\text{s}$.

Reference k_a Values

The ShakeReactor utilizes polycarbonate Erlenmeyer flasks from Corning, for which we have extensively studied oxygen transfer characteristics. **k_a values have been measured across various flask sizes, baffle configurations, fill levels, and shaking speeds (RPM) using a buffered saline solution as a reference medium, see figure 2.**

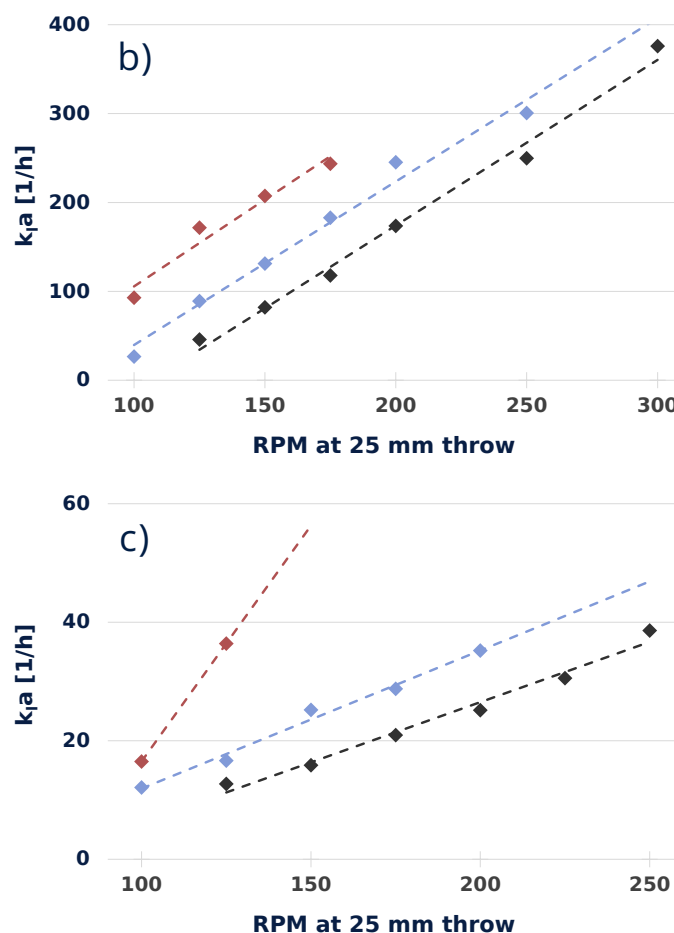
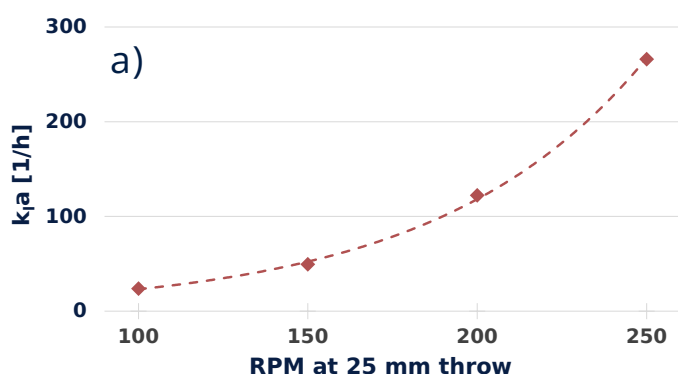


Figure 2 — k_a as a function of RPM for 100 mL —, 200 mL — and 300 mL — fill in a) 500 mL baffled-, b) 1 L baffled- and c) 1 L non-baffled -Corning polycarbonate Erlenmeyer flasks.

Practical recommendations

Ensuring sufficient oxygen availability is essential for reproducible and scalable shake flask cultivations. Below are some practical recommendations:

1. Reduce Liquid Volume

Reducing culture volume increases the gas-liquid surface area to volume ratio, which increases k_a . However, going too low can lead to out-of-phase behaviour, where the liquid does not move in phase with the shaking, significantly reducing oxygen transfer — **15-20% fill volume** is recommended.

2. Ensure filter allows adequate oxygen transfer

If the filter's k_w is too small, it will lead to a drop in headspace oxygen concentration during cultivation. **Avoid cotton plugs, aluminium foil, or solid caps**, as they restrict gas exchange. Instead, use thin filter based flask closures.

3. Minimize Foam Formation

Excessive foaming creates a layer which insulates the culture from oxygen. Variation in foaming between runs and strains can lead to problems with reproducibility. Use small amounts **antifoam agents** in case of excessive foaming.

4. Use Baffled Flasks

Baffled flasks create turbulence and splashing, which increases gas-liquid surface area, in turn increasing oxygen transfer rates. They are particularly useful for **high-density cultures**, but can also be useful for mammalian cells.

5. Estimate Oxygen Transfer Using Reference Values

For researchers without access to oxygen monitoring, **published k_a reference tables** provide an alternative method to estimate oxygen transfer under specific shaking conditions. While these estimates may have a larger margin of error, they serve as a valuable tool for **identifying major oxygenation issues**.

6. Monitor Oxygen Transfer in Real Time

The most effective way to ensure optimal oxygen availability is through **real-time dissolved oxygen (DO) monitoring**. Online oxygen sensors allow researchers to **detect oxygen limitations as they occur**, providing immediate feedback for process optimization. This approach enables a more precise understanding of oxygen transfer dynamics and supports data-driven decision-making in bioprocess development cultures.