

# Volumetric Mass Transfer Coefficient ( $k_L a$ )

AppNote by **Kuhner** shaker



Keebler, M.V.<sup>1</sup>, Schulte, A.<sup>2</sup>, Laidlaw, R.D.<sup>1</sup>, Anderlei, T.A.<sup>3</sup>

1. Kuhner Shaker, Inc., San Carlos, CA, United States
2. Kuhner Shaker GmbH, Herzogenrath, Germany
3. AG Kuhner, Birsfelden, Switzerland

The transfer of gaseous compounds into and out of the liquid is of key importance for any shaken bioprocess. The volumetric mass transfer coefficient ( $k_L a$ ) is a key parameter that defines the limits of this transaction and is defined by the shaking parameters and the properties of the liquid, gas, and shaking vessel. Here we introduce the  $k_L a$  parameter in the context of the different variables that have been used to define it and discuss how the properties of the liquid and the shaking vessel itself can impact the  $k_L a$ .

## What is $k_L a$ ?

The volumetric mass transfer coefficient ( $k_L a$ ) is a parameter that determines the rate at which a gaseous compound (e.g.  $O_2$  or  $CO_2$ ) can transfer between the gas phase and the liquid phase. The ' $k_L$ ' represents the rate of molecular diffusion through the gas-liquid interface and the ' $a$ ' represents the area of this interface per liquid volume [1]. Together, these parameters form the  $k_L a$ , which is often described in units per time and can be utilized to define the limits of any given bioprocess with respect to gas transfer (e.g.  $OTR_{max}$ ). This information will help determine if a bioprocess is oxygen limited by design, which can be instrumental when optimizing any aerobic bioprocess in a shaken vessel [2-5].

## How is the $k_L a$ defined?

The  $k_L a$  is defined by the properties of the vessel, the properties of the liquid, and the shaking parameters. Several models have been developed to theoretically estimate the  $k_L a$  in a shaken bioreactor [1,6-9] and these models can be utilized to compare different scales within a geometrically similar vessel. The equations for these models often utilize the following key variables: vessel diameter ( $d$ ), shaker diameter ( $d_0$ ), shaker frequency ( $n$ ), filling volume ( $V_L$ ), diffusion coefficient for oxygen ( $D_{O_2}$ ), the kinematic viscosity ( $\nu$ ), and the gravitational acceleration ( $g$ ). Dimensional analysis of these variables yields the following equation, which includes a constant ( $C$ ) and the following dimensionless numbers: Froude Number, Volume Number, Geometric Number, Galilei Number, and Schmidt Number [6].

$$k_L a \cdot \left(\frac{\nu}{g}\right)^{\frac{1}{3}} = C \cdot \left(\frac{n^2 \cdot d_0}{g}\right)^{\alpha} \cdot \left(\frac{V_L}{d^3}\right)^{\beta} \cdot \left(\frac{d_0}{d}\right)^{\gamma} \cdot \left(\frac{d^3 \cdot g}{\nu^2}\right)^{\delta} \cdot \left(\frac{\nu}{D_{O_2}}\right)^{\epsilon}$$

Froude Number
Volume Number
Geometric Number
Galilei Number
Schmidt Number

The exponents ( $\alpha$ - $\epsilon$ ) represent the influence of each number and are each individually and empirically determined using data collected from an off-gas analyzer like the Kuhner TOM.

The simplified equation below can be utilized for calculating the  $k_L a$  in a large-scale cylindrical vessel with a flat-bottom surface (2-200L) [6].

$$k_L a = 1.06 \cdot 10^{-3} \cdot d^{4.3} \cdot n^{2.12} \cdot V_L^{-1.2} \cdot \nu^{-0.21} \cdot D_{O_2}^{0.12} \cdot g^{-0.51}$$

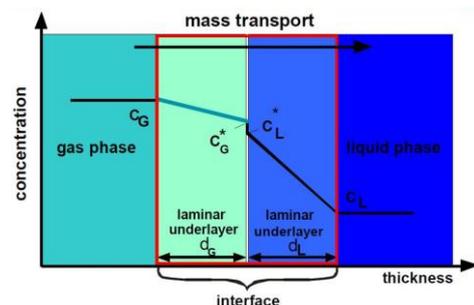
Vessel Dia.
Shake Freq.
Filling Vol.
Viscosity
O<sub>2</sub> Diffusion
Gravity

Similar equations have been described for micro-titer plates [7] and shake flasks [8-9]. The following is an example of an equation that can be used to calculate the  $k_L a$  in a shake flask [8]:

$$k_L a = 0.5 \cdot d^{2.03} \cdot n \cdot V_L^{-0.89} \cdot \nu^{-0.24} \cdot D_{O_2}^{0.5} \cdot g^{-0.13} \cdot d_0^{0.25}$$

Vessel Dia.
Shake Freq.
Filling Vol.
Viscosity
O<sub>2</sub> Diffusion
Gravity
Shaker Dia.

The rate at which molecules (e.g.  $O_2$ ) will transfer between the gas and the liquid is a function of the  $k_L a$  and the concentration gradient between the saturated gas/liquid interface and the bulk phase (illustrated below).



We can assume that the diffusion resistance of the laminar underlayer for the gas phase (mint green) is negligible and so the kinetics of this transport will mostly be defined by the transition across the laminar underlayer of the liquid phase. This assumption allows us to relate the  $OTR$  and  $OTR_{max}$  to the  $k_L a$  using the following equations [1, 6]:

$$OTR = k_L a \cdot (c_L^* - c_L)$$

$$OTR_{max} = k_L a \cdot L_{O_2} \cdot p_{O_2}$$

# Volumetric Mass Transfer Coefficient ( $k_L a$ )

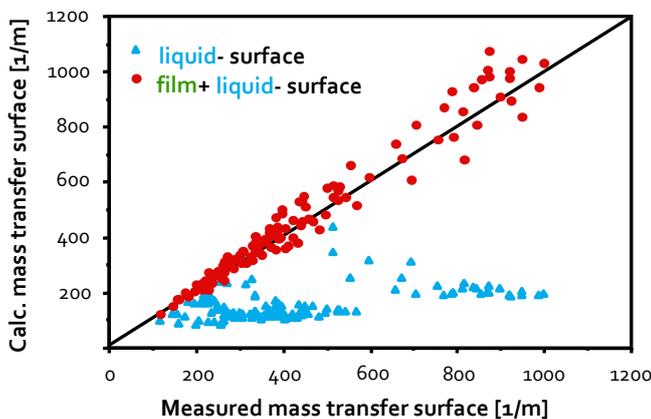
AppNote by **Kühner** shaker



The second equation for  $OTR_{max}$  assumes that the concentration of  $O_2$  in the liquid has been completely depleted (i.e.  $O_2$  limited) and is dependent on the  $O_2$  solubility ( $L_{O_2}$ ) and the  $O_2$  partial pressure in the gas phase ( $p_{O_2}$ ). The  $L_{O_2}$  of  $H_2O$  is approximately  $1.18 \text{ mmol L}^{-1} \text{ bar}^{-1}$  at  $30^\circ\text{C}$  (decreasing with temperature) and will vary with the chemical composition of the liquid (10). The  $p_{O_2}$  is heavily reliant on the aeration of the vessel, which will significantly affect the  $OTR_{max}$  (11). For a further discussion of  $OTR_{max}$ , please review the Kühner AppNote: Maximum Oxygen Transfer Capacity.

## Where does most of the mass transfer take place?

When shaking under optimal conditions (please see the Kühner AppNote: "How to calculate the minimum shaking frequency in microtiter plates"), a mixing angle is formed, and the bulk of the liquid will circulate along the wall of the vessel. A thin film of liquid will remain on the wall of the vessel following each revolution of the wave and this is where a significant fraction of gas transfer will take place. This was demonstrated by showing that theoretical calculations of mass transfer align with actual measurements of mass transfer only when the film of the liquid is factored into the model [Figure 1][12].

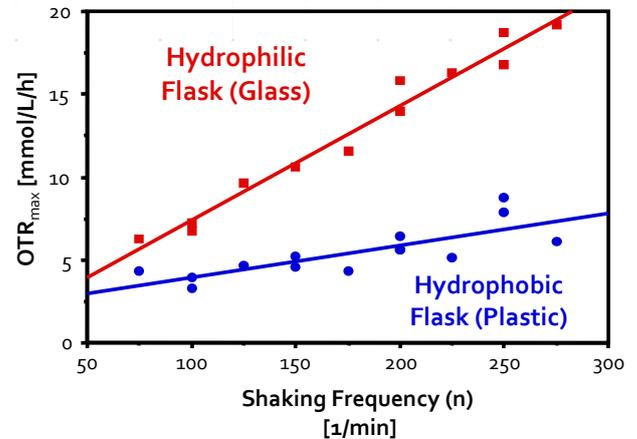


**Figure 1. Contribution of the liquid film to mass transfer**

Comparison of the theoretical and measured mass transfer area in a 250mL shake flask for the bulk of the liquid (blue) and bulk of the liquid plus the liquid film on the walls of the vessel (red). Data from Maier and Büchs (2001) [12].

## How does the vessel material impact $k_L a$ ?

Different chemical properties of the vessel (e.g. hydrophobicity) will influence the development of this film, which will directly affect the  $k_L a$  and  $OTR$  [Figure 2]. In general, vessels that are composed of a more hydrophilic material (e.g. glass) will retain a more favorable film of liquid for gas transfer than vessels composed of a more hydrophobic material (e.g. plastic).



**Figure 2.  $OTR_{max}$  for different flask materials.**

Data represent  $OTR_{max}$  as a function of shaking frequency on an orbital shaking platform (50mm diameter). Measurements were made using a 1M sodium sulfite system in a 250mL, non-baffled, glass (red) or plastic (blue) shake flask with a 10% filling volume. Data from Maier and Büchs (2001) [12]

In summary, the  $k_L a$  defines the limit for the transfer of mass between the gas and liquid phase. This parameter has been shown to be very helpful in the scale-up and optimization of bioprocesses [1-5] and is directly impacted by the properties of the vessel, the properties of the liquid, and the shaking parameters. Several models have been described to calculate the  $k_L a$  for different vessels and these equations can help determine the limits of an experiment with respect to gas transfer.

## References:

1. Klöckner, W., Büchs, J. 2012. Advances in shaking technologies. Trends Biotechnol. 30(6): 307-314.
2. Zhang, X., Stettler, M., De Sanctis, D., Perrone, M., Parolini, N., Discacciati, M., De Jesus, M., Hacker, D., Quarteroni, A., Wurm, F.M. 2009. Use of orbital shaken disposable bioreactors for mammalian cell cultures from the milliliter-scale to the 1,000-liter scale. Adv. Biochem. Eng. Biotechnol. 115: 33-53.
3. Zhang, X., Bürki, C.A., Stettler, M., De Sanctis, D., Perrone, M., Discacciati, M., Parolini, N., Hacker, D., Quarteroni, A., Wurm, F.M. 2009. Efficient oxygen transfer by surface aeration in shaken cylindrical containers for mammalian cell cultivation at volumetric scales up to 1000L. Biochem. Eng. J. 45(1): 41-47.
4. Tissot, S., Michel, P.O., Hacker, D.L., Baldi, L., De Jesus, M., Wurm, F.M. 2012.  $k_L a$  as a predictor for successful probe-independent mammalian cell bioprocesses in orbitally shaken bioreactors. N Biotechnol. 29(3): 387-94.
5. Tissot, S., Oberbek, A., Reclari, M., Dreyer, M., Hacker, D.L., Baldi, L., Farhat, M., Wurm, F.M. 2011. Efficient and reproducible mammalian cell bioprocesses without probes and controllers? N Biotechnol. 28(4): 382-90.
6. Klöckner, W., Gacem, R., Anderlei, T., Raven, N., Schillberg, S., Lattermann, C., Büchs J. 2013. Correlation between mass transfer coefficient  $k_L a$  and relevant operating parameters in cylindrical disposable shaken bioreactors on a bench-to-pilot scale. J. Biol. Eng. 7(1): 28.
7. Doig, S.D., Pickering, S.C.R., Lye, G.J., Baganz, F. 2005. Modelling surface aeration rates in shaken microtitre plates using dimensionless groups. Chem. Eng. Sci. 60(10): 2741-50.
8. Henzler, H.J., Schedel, M. 1991. Suitability of the shaking flask for oxygen supply to microbiological cultures. Bioprocess Eng. 7: 123-131.
9. Nikakhtari, H., 2008. Modelling Oxygen Transfer and Aerobic Growth in Shake Flasks and Well-Mixed Bioreactors. The Canadian J. Chem. Eng. 83(3): 493-99.
10. Wilhelm, E., Battino, R., and Wilcock, R.J., 1977. Low-pressure solubility of gases in liquid water. Chem. Rev., 77(2): 219-262.
11. Mrotzek, C., Anderlei, T., Henzler, H., Büchs, J. 2001. Mass transfer resistance of sterile plugs in shaking bioreactors. Biochem Eng J. 7(2): 107-112.
12. Maier, U., Büchs, J. 2001. Characterisation of the gas-liquid mass transfer in shaken bioreactors. Biochem. Eng. J. 7(2): 99-106.