Maximum oxygen transfer capacity in shake flasks

AppNote by Kuhner shaker

Keebler, M.V.[1], Laidlaw, R.D.[1], Anderlei, T.A.[2]

1. *Kuhner Shaker, Inc., San Carlos, CA, United States*
2. *AG Kuhner, Birsfelden, Switzerland*

Shake flasks are a widely used and incredibly useful bioreactor for bioprocess development and scale-up of mammalian and microbial bioprocesses [1-3]. There are several parameters to consider when optimizing a bioprocess in a shake flask, including the conditions of the shaker platform (e.g. shaking frequency and orbital diameter) and the conditions of the shake flask (e.g. flask size and filling volume). The maximum oxygen transfer capacity (OTR_{max}) is a measure of the maximum rate at which oxygen transfers between the gas and the liquid phase in the vessel. Since most cells require oxygen to grow, this is generally viewed as a key predictor for the performance of a shaken bioprocess. Here we review how the OTR_{max} of a shake flask will vary as a function of both shaker conditions and flask conditions.

Measuring OTR_{max} in a shake flask

Several instruments exist for monitoring the OTR in a shake flask, including the RAMOS [4] and the Kuhner TOM (www.kuhner.com). To determine the OTR_{max} in a shake flask, artificial systems are often utilized to simulate metabolic activity from an aerobic organism [5-6]. One system that is commonly used, the sodium sulfite system [7], relies on a chemical reaction between sodium sulfite (Na_2SO_3) and dissolved oxygen (O_2), according to the following reaction:

$$SO_3^{-2} + 0.5\ O_2 \xrightarrow{Co^{2+}} SO_4^{-2}$$

The absolute OTR_{max} reported from a sodium sulfite system will vary depending on the concentration of the sodium sulfite and will require a proportionality factor when used to estimate the OTR_{max} of cell culture media [6-8]. The Kuhner AppNote, “Maximum oxygen transfer capacity”, discusses these proportionality factors in greater detail and includes a lists in the Appendix of several factors that have been reported for different systems and parameters.

The OTR_{max} is a function of the volumetric mass transfer coefficient (k_La), which describes the kinetics of transfer for a gaseous compound at the gas-liquid interface in terms of the chemical diffusion (k_L) and the volumetric surface area between the phases (a). Various models have been used to estimate the k_La in a shake flask and often include the following four key parameters: shaking frequency [1/min], diameter of the shaker orbit [cm], liquid filling volume [mL], and diameter of the flask [mm] [6]. Here we rely on the model described by Meier et al. (2016) [8] to calculate the OTR_{max}, which is also discussed in greater detail within the Kuhner AppNote, “Maximum oxygen transfer capacity”.

OTR_{max} and the orbital diameter of the shaker

The shaking frequency and orbital diameter of the shaker platform synergize to create a centrifugal force that pushes the liquid up the side of the vessel. As a result, the surface area of the gas-liquid interface increases, which in turn will increase the k_La and OTR_{max}. The data in Figure 1 illustrate this relationship by showing an increase in OTR_{max} of a shake flask with an increase in the shaking frequency and orbital diameter of the shaker. One of the advantages of a Kuhner shaker is that the user has the opportunity to adjust the orbital diameter of the shaker platform. As illustrated in Figure 1, this would allow the user to adjust the frequency of the shaker without changing the OTR_{max}.

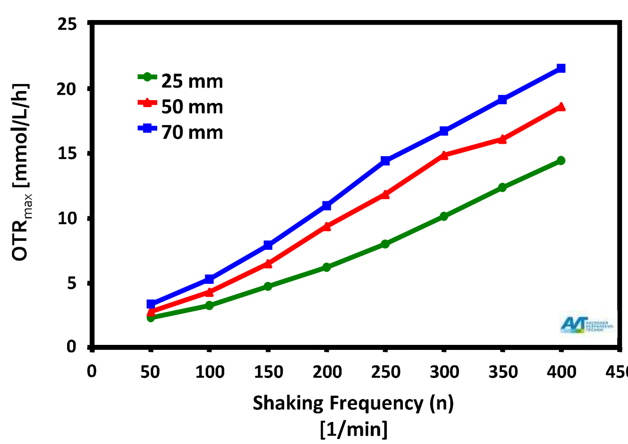

Figure 1. OTR_{max} for different orbital diameters.
Data represent OTR_{max} as a function of shaking frequency for orbital shaking diameters of 70mm (blue), 50mm (red), 25mm (green). The liquid filling volume was 10% of a 250mL, non-baffled shake flask. Measurements were made using a 1M sodium sulfite system following the protocol outlined in [5].

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OTR_{max} and the size of the flask

The chosen size of the shake flask and the fraction of total volume occupied by liquid (filling volume) will also impact the k_La and OTR_{max}. Since the volumetric surface area of smaller shake flasks is relatively greater than that of larger shake flasks, these smaller flasks will observe a larger k_La (data not shown) and OTR_{max} (Figure 2).

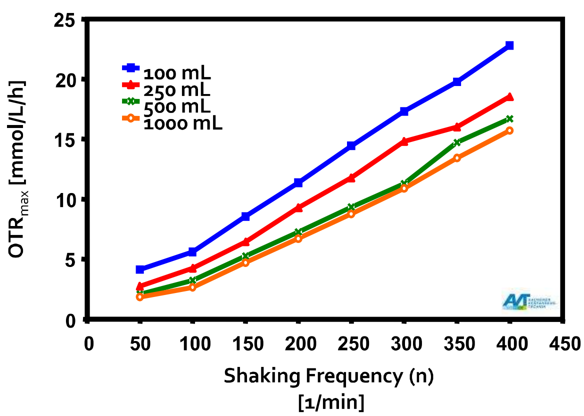

Figure 2. OTR_{max} for flasks of different sizes.

OTR_{max} is shown as a function of shaking frequency on an orbital shaker platform with a 50mm shaking diameter for flask sizes of: 100mL (blue), 250mL (red), 500mL (green), and 1L (orange). The liquid filling volume in each non-baffled flask was 10%. Measurements were made using a 1M sodium sulfite system following the protocol outlined in [5].

It has been reported that the ventilation and neck diameter of a shake flask can limit the rate of aeration and oxygen exchange with the headspace (volume sitting above the liquid), which would also impact the OTR_{max} [9]. While the data shown in Figure 2 have neutralized any effect of neck diameter and ventilation by directly aerating into the vessel at a high flow rate, optimizing the neck diameter and ventilation of the vessel could still lead to better results when scaling up in a shake flask. Some companies offer shake flasks with a wider neck diameter and greater ventilation to specifically address this issue.

OTR_{max} and the filling volume

Another factor that will define the volume of the headspace in the shake flask, and the corresponding rate of oxygen transfer between the gas and the liquid, is the volume of the liquid itself (filling volume).

Given that the k_La is a function of filling volume, and that the k_La defines the OTR_{max}, greater filling volumes will result in a much lower OTR_{max} than smaller filling volumes. This will result in less oxygen available for each cell in the liquid and is one of the reasons why scaled up processes may experience lower yields. Figure 3 illustrates this relationship for a 250mL shake flask with filling volumes of 4%, 10%, and 20%.

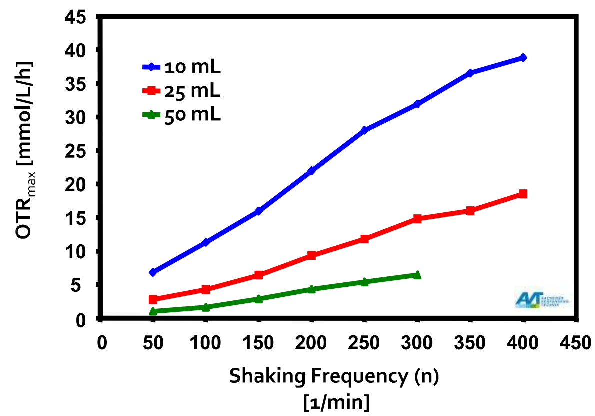

Figure 3. OTR_{max} for different fill volumes.

Data represent OTR_{max} as a function of shaking frequency on an orbital shaker platform with a 50mm orbital diameter. The liquid filling volumes include: 10mL (blue), 25mL (red), and 50mL (green) in a 250mL, non-baffled flask. Measurements were made using a 1M sodium sulfite system following the protocol outlined in [5].

In summary, the OTR_{max} can be instrumental when planning or optimizing the conditions for an aerobic culture in a shake flask. A few key parameters to consider include: shaker frequency, orbital diameter, flask size, and filling volume.

References:

1. Van Suijdam, J.C., Kossen, N.W.F., Joha, A.C. 1978. Model for oxygen transfer in a shake flask. Biotechnol. Bioeng. 20: 1695-1709.
2. Henzler, H.J., Schedel, M. 1991. Suitability of the shake flask for oxygen supply to microbial culture. Bioprocess. Eng. 7: 123-131.
3. Veljkovic, V.B., Nikolic, S., Lazic, M.L., Engler, C.R. 1995. Oxygen transfer in flasks shaken on orbital shakers. Hem. Ind. 49(6): 265-272.
4. Anderlei, T.A., Zang, W., Papaspyrou, M., Büchs, J. 2004. Online respiration activity measurement (OTR, CTR, RQ) in shake flasks. Biochem. Eng. J. 17(3): 187-194.
5. Maier, U., Büchs, J. 2001. Characterisation of the gas-liquid mass transfer in shaken bioreactors. Biochem. Eng. J. 7(2): 99-106.
6. Maier, U., Losen, M., Büchs, J. 2004. Advances in understanding and modeling the gas-liquid mass transfer in shake flasks. Biochem. Eng. J. 17(3): 155-167.
7. Linek, V., Vacek, V. 1981. Chemical engineering use of catalyzed sulphite oxidation kinetics for the determination of mass transfer characteristics of gas-liquid contractors. Chem. Eng. Sci. 36(11): 1747-176
8. Meier, K., Klockner, W., Bonhage, B., Antonov, E., Regestein, L., Büchs, J. 2016. Correlation for the maximum oxygen transfer capacity in shake flasks for a wide range of operating conditions and for different culture media. Biochem. Eng. J. 109: 228-235.
9. Tanaka, H., Ishikawa, H., Takagi, Y. 1992. A new scale-up method based on the effect of ventilation on aerated fermentation systems. J. Ferm. Bioeng. 72(3): 204-209.

Acknowledgements:

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*AVT – Biochemical Engineering, RWTH Aachen University