Maximum oxygen transfer capacity

AppNote by Kuhner shaker

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Oxygen is an essential nutrient for all aerobic organisms and knowing the maximum oxygen transfer capacity (OTR_{max}) of your cultivation system is critical for experimental planning and optimization. Here we discuss the key parameters that define the OTR_{max} and illustrate how these parameters can be used to theoretically estimate the OTR_{max} for any aerobic system. We also discuss the use of proportionality factors to relate empirical measurements of the OTR_{max} from a sulfite system to any biological system.

What is the OTR_{max} and why is it important?

The OTR_{max} defines the maximum capacity for oxygen transfer between the gas and the liquid inside a shaking vessel [1]. Once gaseous oxygen has dissolved into the culture media, it is available as a nutrient for living organisms to consume. The rate at which dissolved oxygen is consumed in the liquid by aerobic organisms is referred to as the oxygen uptake rate (OUR). When the OUR is greater than the OTR_{max}, the metabolic demand for oxygen is not being met and growth of the culture will be limited by the OTR_{max} [1-5]. This phenomenon, known as 'oxygen limitation', can be addressed by optimizing the shaking conditions to ensure that the OTR_{max} is greater than the OUR requirements of the culture.

How is the OTR_{max} calculated?

The key parameters that will impact the OTR_{max} of any shaken process include the: (1) osmolality of the solution, (2) shaking frequency, (3) filling volume, (4) shaker diameter, and (5) vessel diameter. Meier et al. (2016) describe a model for the calculation of OTR_{max} in shake flasks, utilizing the equation below with the above mentioned parameters [5]. The equation also includes a variable for the absolute pressure in the flask (p_R), which is assumed to be equivalent to ambient pressure (1 bar), and the mole fraction of O_2 in the ambient air (y_{O_2}), which is assumed to be ~0.21. This equation can be utilized as a valuable tool to calculate and compare the OTR_{max} values for different media under the same shaking conditions. For instance, if a user were growing Chinese Hamster Ovarian (CHO) cells in 40mL of ProCHO™ 5 media, by shaking in a 250mL Erlenmeyer (non-baffled) flask at 150rpm with a 50mm orbital diameter, they should expect an OTR_{max} value of ~10.8 mmol/L/h.

If the user then decided to switch to the ExpiCHO™ Stable Production Medium and maintain the same shaking conditions, we would only need to change the osmolality value in the equation from 0.350 Osmol/kg [6] to 0.295 Osmol/kg [7]. This would increase the OTR_{max} to ~11.0 mmol/L/hr. By adjusting the values for the different parameters in this equation, the user can easily predict the OTR_{max} across different process conditions. Table 1 lists a few of these calculations for different media. These predictions can also be empirically confirmed using an online, off-gas analyzer like the Kuhner TOM (www.kuhner.com).

Converting OTR_{max} between systems

The sodium sulfite system is a useful tool for mimicking biological activity in a shaking vessel [4]. The chemical reaction between sulfite (SO_3) and dissolved oxygen (O_2) is catalyzed with cobalt to produce sulfate (SO_4) and consume oxygen. These chemical systems have been widely used to study how the OTR_{max} varies with respect to different shaking conditions [4,5,8,9]. Many users still rely on these data sets for bioprocess development and optimization, and the OTR_{max} values from these chemical systems can be easily converted to any biological media using a proportionality factor. Since the equation listed below is specific to Erlenmeyer flasks, this approach of relating empirical measurements from a chemical system to a biological system could be useful for other vessel types or to capture additional variables that may not be factored into the equation.

These proportionality factors usually range between 1 to 3 and will likely vary with media composition and shaking conditions (Figure 1). Table 2 (Appendix) lists a few examples of proportionality factors for different media and process conditions.

$$OTR_{max} = 3.72 \times 10^{-4} \times \underbrace{\mathrm{Osmol}^{0.05}}_{\text{Osmolality (Osmol kg}^{-1})} \times \underbrace{n^{\left(1.18-\frac{\mathrm{Osmol}}{10.1}\right)}}_{\text{Shake Frequency (RPM)}} \times \underbrace{V_L^{-0.74}}_{\text{Filling Vol. (mL)}} \times \underbrace{d_0^{0.33}}_{\text{Shaking Dia. (cm)}} \times \underbrace{d^{1.88}}_{\text{Flask Dia. (mm)}} \times p_R \times y_{O_2}$$

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Table 1. Calculated OTR_{max} values for different media.

OTR_{max} calculations for different media osmolality and shaking conditions, based on equation described in Meier et al., (2016) [5].

Media	OTR_{max} (mmol/L/h)	Constant	Osmolality (Osmol/kg)	Shake Frequency (RPM)	Filling Volume (mL)	Shaker Diameter (cm)[a]	Flask Diameter (mm)	Absolute Pressure (bar)	O_2 mole fraction
ProCHO 5 Media	10.8	3.72E-04	0.350	150	40	5	85	1	0.21
ExpiCHO™ Stable Production Medium	11.1	3.72E-04	0.295	150	40	5	85	1	0.21
ExpiCHO™ Expression Medium	11.0	3.72E-04	0.295	150	40	5	85	1	0.21
Expi293™ Expression Medium	11.0	3.72E-04	0.285	150	40	5	85	1	0.21
TB-Medium	62.5	3.72E-04	0.500	300	10	5	85	1	0.21
LB-Medium	69.8	3.72E-04	0.240	300	10	5	85	1	0.21
1M Sodium Sulfite System	24.4	3.72E-04	2.300	300	10	5	85	1	0.21
0.5M Sodium Sulfite System	41.7	3.72E-04	1.300	300	10	5	85	1	0.21

[a] Average width of 250mL, unbaffled flask.

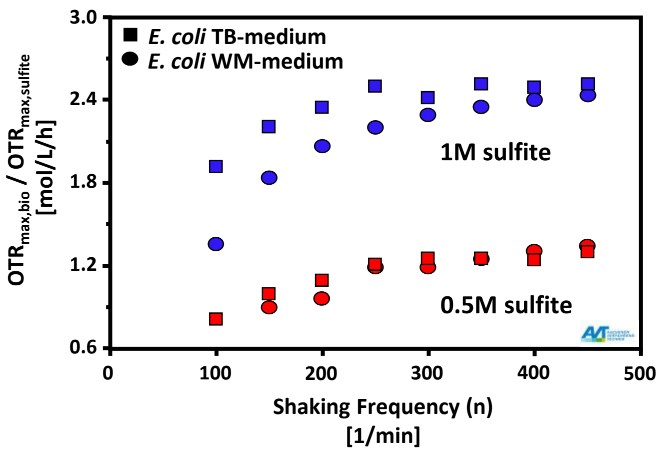

Figure 1. Proportionality of OTR_{max} between culture media and sodium sulfite systems.

Data represent ratio of the OTR_{max} for *E. coli* in terrific broth (TB) media (squares) or Wilms-MOPS (WM) media (circles) with the OTR_{max} measured with a 1M sulfite system (blue) or a 0.5M sulfite system (red) as a function of shaking frequency. Data used with permission from Meier et al., (2016) [5].

In summary, the OTR_{max} defines the capacity for oxygen transfer in a system and is an important parameter for the scaling or optimization of any aerobic process. The model described by Meier et al., 2016 [5] can be used to calculate the theoretical OTR_{max} of any system and this can be empirically validated using an online, off-gas analyzer like the Kuhner TOM (www.kuhner.com). Many OTR_{max} measurements have come from non-biological systems and are often used to compare the performance of different shaking conditions. These OTR_{max} measurements can be easily translated into any biological system using a proportionality factor for that system. Using these tools to determine the OTR_{max} of a system will help ensure that the OUR of a system is never greater than the OTR_{max}.

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Appendix

Table 2. Proportionality factors to convert the OTR_{max} measurements from a sulfite system to a biological media.
Proportionality factors relating OTR_{max} measurements between different culture media and different sodium sulfite systems are listed along with the associated culture parameters.

[Sulfite]	Orbit (mm)	Shaking Frequency (RPM)	Shaking Vessel	Fill ing Volume (%)	Cell Type	Media	Proportionality Factor	Literature
1M	50mm	50-500rpm	Shake flask (250mL)	4-16	*P. pastoris*	YPG	2.8	[4]
1M	50mm	200-350	Shake flask (250mL), MTP (24w)	4-20, 21-29	*T. reesei*	Complex media	1.9 to 2.4	[10]
1M	50mm	300	Shake flask (500mL)	8	*C. glutamicum*	Complex media	1.8	[11]
1M	50mm	300	Shake flask (500mL)	8	*H. polymorpha*	YNB	~1.52	[12, 11]
1M	50mm	350	Shake flask (250mL)	4	*E. coli* (BL21-DE3)	Terrific Broth	~1.97[a]	[13, 10]
1M	50mm	350	Shake flask (250mL)	4	*E. coli* (BL21-DE3)	Wilms-MOPS	~1.59[a]	[13, 10]
1M	50mm	350	Shake flask (250mL)	4	*E. coli* (K12 and BL21)	Wilms-MOPS	~1.47[a]	[14, 10]
1M	50mm	350	Shake flask (250mL)	4	*A. adeninivorans* (LS3)	SYN6-PO_4	~1.43[a]	[15, 10]
0.5M	25mm; 50mm	450; 250, 300	MTP (48w)	29	*H. polymorpha*	YNB	~1.35[a]	[8]
0.5M	50mm	350	Shake flask (250mL)	4	*B. licheniformis*	V3 MOPS	1.84	[16]
0.5M	50mm	320 and 240	Tube (15mL), Shake flask (250mL)	6, 4	*H. polymorpha*	YNB	1.5	[17]
0.5M	50mm	300	MTP (96w) w/System Duetz	21	*P. putida*	Mineral medium	~1.38	[18]
0.5M	25mm	250	Shake flask (250mL)	12	*P. putida*	Mineral medium	~1	[9]
0.35M	50mm	350	Shake flask (250mL)	12	*C. glutamicum* (DM1730)	Complex media	~1.1	[19]

[a] OTR_{max} values utilized for these proportionality factors were separately reported, though measured under the same conditions