Cellexus

White Paper:

Volumetric Mass Transfer Coefficient in the CellMaker bioreactor bags

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Introduction

For aerobic processes, the gassing system within the bioreactor must be designed to ensure sufficient supply of oxygen to the cultivated organisms. Due to its low solubility in water, oxygen supply is commonly a limiting factor for many fermentation and cell culture bioprocesses. Therefore, it is vital to characterize the oxygen transfer rate (OTR) of a given bioreactor. This transfer rate can be defined according to the Equation 1:

(1) $OTR = k_L a\ (DO^* - DO)$

This contains the volumetric mass transfer coefficient ($k_L a$) as the driving parameter which can be separated in to the mass transfer coefficient k_L and the volumetric interfacial gaseous-liquid surface area *a*. Since it is extremely difficult to measure the k_L and *a* values individually, both are measured in the coupled form: $k_L a$.

In this application note we describe measurement of $k_L a$ values on the CellMaker Plus system. Alongside the novel airlift suspension design, the CellMaker Plus model can measure pH and dissolved oxygen (DO_2) levels for each of our single-use bioreactors. For this application note, the SOP used to determine the $k_L a$ was the 'gassing-out method' based on the DECHEMA guidelines – an expert group in bioreactor characterizations (Recommendations for process engineering characterisation of single-use bioreactors and mixing systems by using experimental methods, DECHEMA, 2016, ISBN 9783897461710).

Experimental Conditions

A series of experiments was performed in the CellMaker Plus 8L and 50L single-use bioreactor systems for a varied levels of air input and media volumes. The experimental conditions were as follows:

Medium:	0.5x PBS Solution in Deionised Water
Temperature:	37°C
Purge Gas:	Nitrogen
pH:	7

Compressed air was used instead of standard fan pumping of atmospheric air to ensure no pulsating effect on flow. Furthermore, compressed air ensured immediate flowrates of target air flow were achieved subsequent to the nitrogen purging without the delay caused by the internal CellMaker air pump speed ramping to the desired flow rate.

Method

The k_La measurements were conducted based on the SOP published by DECHEMA with modifications. The relative 100% oxygen saturation was measured after the liquid media was sparged with air for at least 20 minutes. Nitrogen flow at 2L/min was then used to purge oxygen from the liquid media and the DO_2 level was monitored continuously. Once the DO_2 value was stable below 2%, the nitrogen flow was stopped. Immediately, the gas in the headspace of the bioreactor bag was removed by manually squeezing the gas out. The airflow was initiated at the stated rate with the DO_2 value monitored until $\%DO_2$ reached 97%. The measurements were performed in the CellMaker Plus Single Use Bioreactor bags 8L and 50L models filled with 5L and 30L of 0.5x PBS, respectively.

The DO_2 values from range 10-90% are then converted using the following Equation 2 and plotted at new y-axis as a function of time (Figure 1).

$$(2)\ \ln\left(DO^*_{max} - DO(t)_{exp}\right)$$

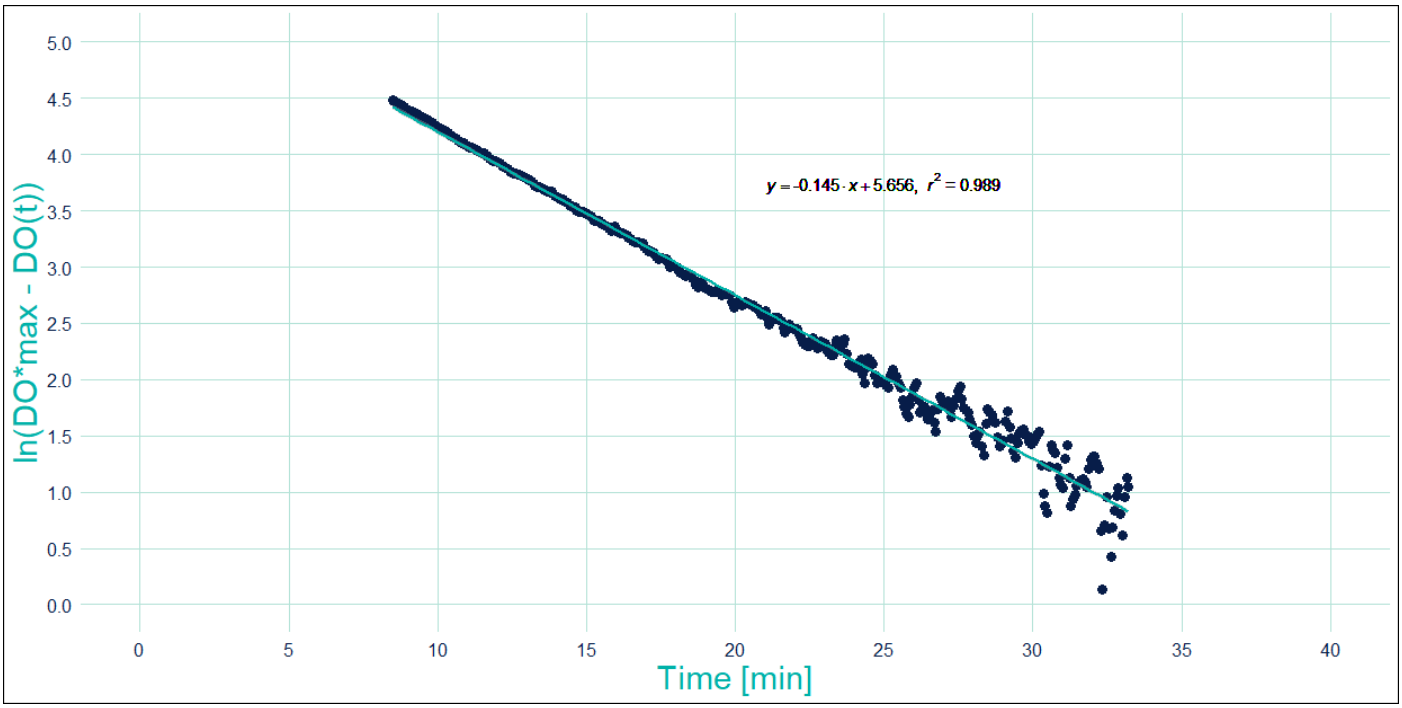

Figure 1. Determination of the k_La value in range of 10 to 90% of max dissolved oxygen in the CellMaker 8L bioreactor bag using airflow of 0.2L/min.

The k_La values of individual measurements were obtained by calculating the best fit curve for each flow rate. In the Figure 1, the k_La = -1/-0.145. The k_La for each condition was calculated as an average of 3 readings, as exampled for air flow of 0.2L/min in 5L of media in the Equation 3.

$$(3)\ \ k_La\left(0.2\ L\ min^{-1}\right) = \frac{1}{n}\sum_{i=1}^{n} k_La_i = \frac{8.688\ hr^{-1} + 11.844\ hr^{-1} + 8.718\ hr^{-1}}{3} = 9.75\ hr^{-1}$$

Results

Varied Flowrates – 8L Enclosure (5L Volume)

At a fixed volume of 5L of media, the relationship of k_La and air flow rate was measured. Figure 2 shows a set of representative experiments where the bag is purged with nitrogen until it reaches <10% DO_2. From there the bag is aerated at chosen flowrate (0.2 - 8L/min) until the DO_2 levels return to 90% of maximum saturation.

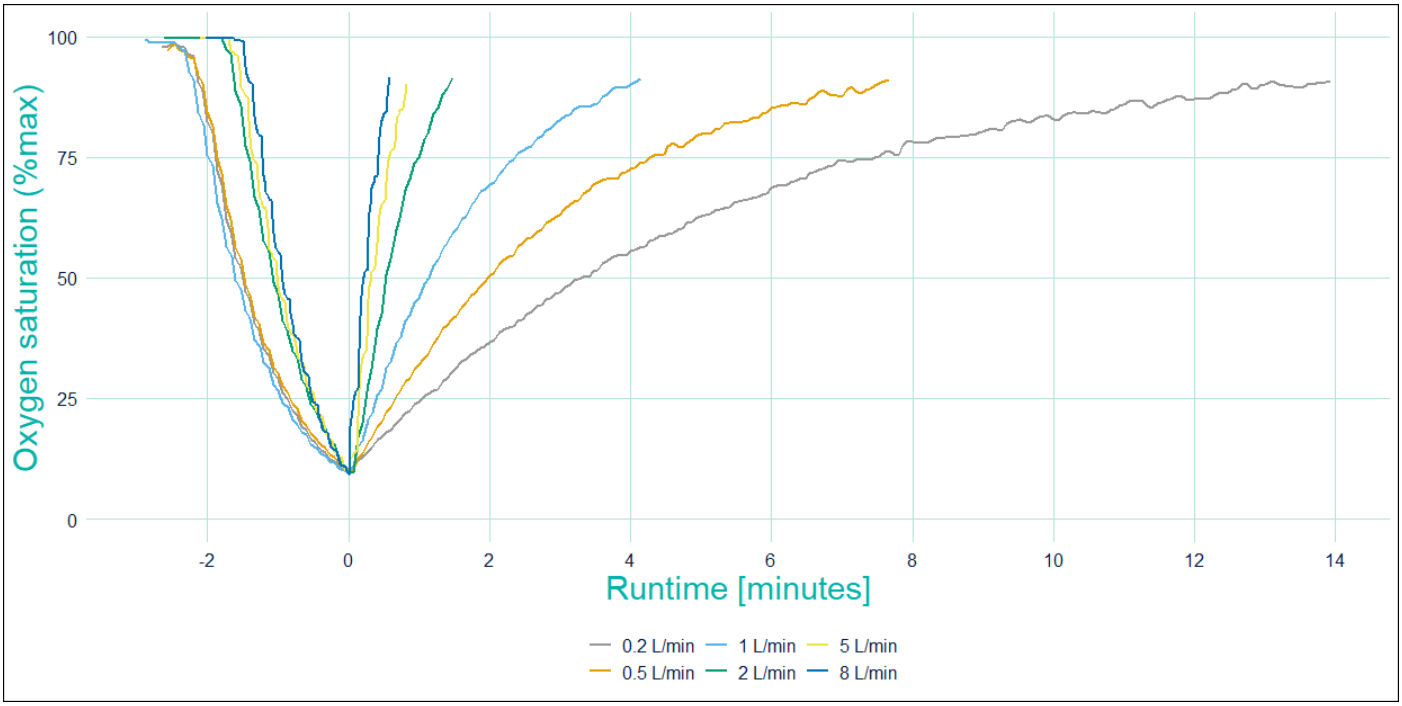

Figure 2. A representative graph of k_La measurement by degassing method in 5L of media in an 8L CellMaker bioreactor bag. The oxygen was depleted by sparging of pure nitrogen through the bag. The 0 minutes mark represents the time when the DO_2 reached 10%, after the air flow was resumed, and the measurement begun. The curve where the time is below 0 represents nitrogen sparging from saturation to DO_2 below 10%. The traces below 10% DO_2 was removed for presentation clarity.

First, we selected the most commonly used fermentation volume utilised in the CellMaker Plus 8L system and filled the bag with 5L of the buffer. The k_La values were measured at a varied flow rate from 0.2L/min (achievable in the CellMaker Low Flow) to 8L/min approaching the maximum aeration rates of the CellMaker Plus model. The tested conditions spanned the ratio of the sparged gas volume to the media volume per minute (VVM) from 0.04 to 1.8. The results are summarised in Table 1.

Flow rate (L min^{-1})	0.2	0.5	1	2.5	5	8
kLa value ($hour^{-1}$)	9.44	20.98	35.7	102.06	157.83	224.26
VVM*	0.04	0.1	0.2	0.5	1	1.8

*VVM – volume of sparged gas per media volume per minute

Table 1. k_La values in the CellMaker Plus 8L system sparged at 0.2 – 8L/min of compressed air.

Varied Media Volumes – 8L Enclosure

One of the key properties of the CellMaker systems is the flexibility of the fermentation media, which can be used in each of the models. The 8L CellMaker systems can support fermentation from 3L of media up to the maximum capacity of 8L. Due to the unique asymmetrical shape of the CellMaker bag, we investigated the relationship between the volume of media with respect to gas flowrate. By keeping the media volume and air flow rate constant in each experiment, the VVM was the only variable parameter (Table 2).

Media Volume (L)	3	5	8
$k_L a$ value ($hour^{-1}$)	61.90	35.70	32.78
VVM*	0.33	0.2	0.125

Table 2. $k_L a$ values for aeration of 3, 5 or 8L of media at 1L/min air flow in the CellMaker Plus 8L system.

As expected, the $k_L a$ values dropped with the reduced VVM. Additionally, the effect of the bag geometry was a significant factor in the aeration of the media, thus the $k_L a$ change with the VVM is not linear in this case.

Varied Flow Rates – 50L Enclosure (30L volume)

The measurements of the $k_L a$ values were repeated using the 50L CellMaker bioreactor bag filled with 30L of media. This is a corresponding level of maximum capacity to the 5L of media in an 8L bioreactor bag. The CellMaker Controller delivers the same air flow rates to all systems, regardless of the connected Enclosure, thus the VVM values we obtained were respectfully lower (Table 3 and Figure 3).

Flow rate ($L\ min^{-1}$)	0.6	1.2	3	6	8
$k_L a$ value ($hour^{-1}$)	5.33	14.13	39.07	66.02	84.15
VVM*	0.02	0.04	0.1	0.2	0.27

Table 3. $k_L a$ values in the CellMaker Plus 50L system sparged at 0.6 – 8L/min of compressed air.

As it could be expected from a higher volume system, the $k_L a$ values were not directly proportional to the values in the 8L system at the same VVM. In fact, the $k_L a$ in the aeration in the 50L system is more efficient, as indicated by approx. double $k_L a$ values per VVM in comparison to the 8L system (e.g. 0.1 VVM and $k_L a$ of 20.98 at 5L and 39.07 at 30L).

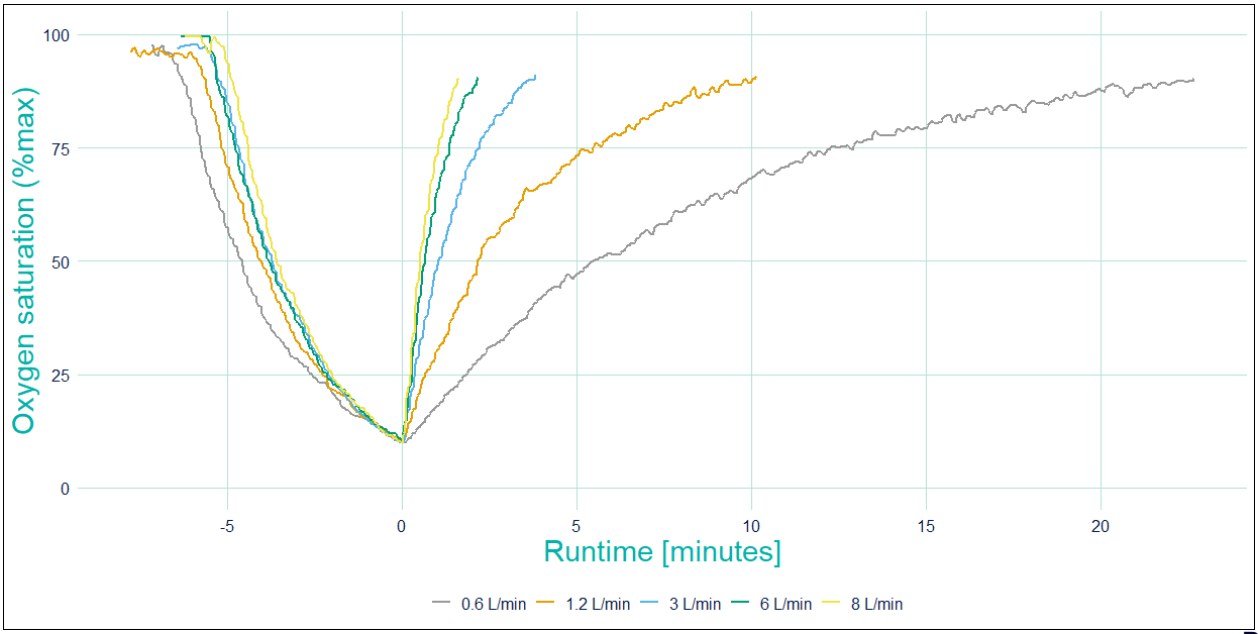

Figure 3. A representative graph of k_La measurement by degassing method in 30L of media in the 50L CellMaker bioreactor bag. The oxygen was depleted by sparging of pure nitrogen through the bag. The 0 minutes mark represents the time when the DO_2 reached 10%, after the air flow was resumed, and the measurement begun. The curve where the time is below 0 represents nitrogen sparging from saturation to DO_2 below 10%. The traces below 10% DO_2 was removed for presentation clarity.

CellMaker aeration performance in comparison to industry leaders

The specific oxygen transfer coefficient (k_La) values are an essential parameter of any bioreactor system, and one of the key parameters impacting the performance of the biological process undertaken in a bioreactor. To meet the requirements of each of the cell types used in bioprocessing, whether these are bacterial, yeast or higher organism cells (e.g. CHO or Hybridoma cells), the bioreactor system needs to provide enough oxygen to fuel the bioprocess. The conventional stirred tank bioreactors are characterised with the most efficient k_La values reaching even 600, however, this is at the cost of high shear forces delivered to the biological system. This trade-off can significantly impact the performance of the cells in the bioreactor and affect the stability of the produced molecules. On the other side of the spectrum are bioreactors specifically designed for cultivation of sensitive cell types, like stem cells or other mammalian cell lines, in which the shear forces are minimised. An example of such system is the wave form, or rocking bed, bioreactor type. However, the k_La of these bioreactors is relatively low, typically in range of 1 to 15h^{-1}.

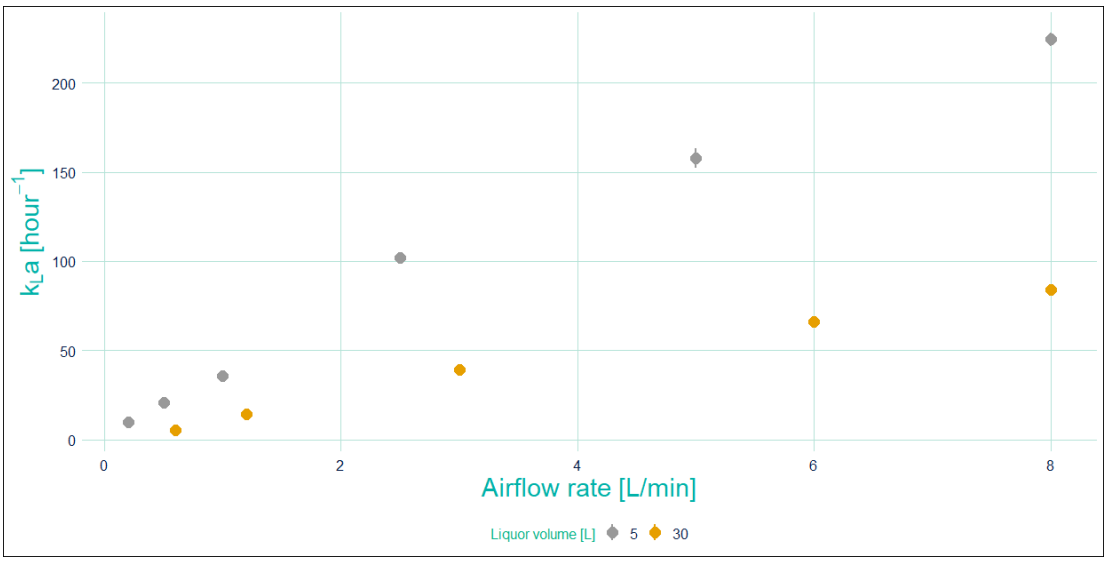

Figure 4. The k_La values achieved by the CellMaker system filled with 5L (grey points) or 30L (orange points) of media and sparged with air at indicated rates. Each point represents an average from 3 independent measurements and the whiskers are standard deviation.

Here we presented the k_La measurements in the CellMaker system, where the mechanical shear stress forces have been eliminated as no mechanical parts are present in the bioreactor chamber. The rising column of gas bubbles provides both mixing, associated with hydraulic shear, as well as gas exchange. Here we present data indicating that the CellMaker airlift bioreactor system can deliver the k_La values in ranges comparable to most other systems on the market. With respect to the low air flow set points (0.2 – 0.5L/min) the concern was that the air bubbles not dispersed by an impeller paddle will not provide sufficient aeration of the cell culture. Our data indicates that the oxygen transfer at these air flow rates is comparable to that of a stirred tank reactor at the same VVM values and same or higher than in a wave form bioreactor, which is well within the requirements of the CHO cell line (approx. $8h^{-1}$) (Figure 4).

The second point of concern was the k_La value at high air flows (5 – 8L/min) required for high cell density microbial cultures. Indeed, the k_La values of the CellMaker are not as high as those achievable with a system equipped with a Rushton-type impeller, however, the significant benefit from the reduction in shear forces in the CellMaker system is not to be ignored. The highest air flows (VVM approx. 1.8), the CellMaker is capable of delivering k_La exceeding $200h^{-1}$ (Figure 4), which is comparable to a standard stirred tank single use system.

Concluding remarks

In this report we presented data obtained in the CellMaker 8L bag, which is the most commonly used bioreactor bag among the CellMaker system user base, as well as the production-scale 50L bag. The patented geometrical shape of the CellMaker bioreactor bags combined with the airlift aeration system make the CellMaker a unique type of a fermentation system. The data presented here place the CellMaker ahead of other low mechanical sheer systems in terms of the capacity to oxygenate the cultivated cells. In fact, at the high VVM rates, the CellMaker loses out only to the stirred tank systems with the highest k_La marks. Unlike many other systems, the CellMaker is very easy to assemble and operate, even at the 50L scale. The single-use nature of the system allows for smooth change of bioprocesses without the risk of cross-contaminating individual fermentation batches and the general design of the CellMaker makes it suitable for both microbial and eukaryotic cell culture while protecting the cells and the bioproduct from excessive damage from shear stress. Notably, the data presented in this report have been obtained while using atmospheric air (21% oxygen, 78% nitrogen, 1% other gases) only. All CellMaker fermentation systems include a digitally controlled, independent inlet for compressed oxygen, or other gas mixture, which can be used to deliver additional oxygen to the fermenter. Thus, the maximum achievable k_La values will depend on the sum of the gases supplied to the system and can be significantly higher than presented here.

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