White Paper

Efficiency of liquid mixing and volumetric mass transfer coefficient in the CellMaker bioreactor systems

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In partnership with: cpi & IBioIC

The most common reasons to move a fermentation process to a bioreactor are to provide high volume fermentation with a uniform, precisely controlled environment and even distribution of substrates. Maintaining homogeneity of all the contents of a bioreactor (cells, metabolites, nutrients, etc.) influences process control and is essential for maintaining optimal conditions. At Cellexus, we see this as a particularly important factor of our innovative airlift bioreactor design.

The CellMaker bioreactor bag does not contain mechanical devices to mix media. Instead, the unique shape of the bag is engineered to provide uniform mixing, and efficient gas and nutrient exchange, using directed flow of gas bubbles through the liquid.

The bag geometry was designed to optimise mixing of the fermentate by providing a circular motion from the conical bottom part of the bag and continuing into the wider top section, where the liquid can circulate and prevent settling of the biological material at the bottom of the bioreactor. The shape of the bioreactor bag also has a significant impact on the volumetric mass transfer coefficient (k_La). k_La is an essential parameter in all aerobic fermentation processes, as it describes how well oxygen can be delivered to the cells in the container.

In this study, we measured efficiency of mixing and oxygenation in the full range of CellMaker bioreactor bags. The CellMaker bioreactors are available in working volumes from 1.5L to 50L. The most quoted benefit of using this bioreactor is the ease of bioprocess scaling.

When up-scaling fermentation, the VVM (Volume of sparged air to Volume of liquid per Minute) is the parameter allowing to estimate the performance of the system based on air sparging. It is particularly useful in the CellMaker systems, as the air flowrate is the principle means of both mixing and aerating the media. By comparing the desired VVM to the volume of fermentate in the bioreactor bag (Figure 1), it is possible to calculate the approximate air flowrate needed to maintain the efficiency of the bioprocess. However, the relationship between the VVM and k_La, is not always directly proportional. The results presented here provide an overview of mixing and aeration efficiency, which can be expected while using the CellMaker and scaling up fermentation.

Mixing time determination

Experimental conditions

A series of experiments was performed in the CellMaker Plus 4L, 8L and 50L bioreactors at input air flow rates from 0.5 to 10 litres per minute (L/min). The corresponding VVM values are presented in Figure 1. The experimental conditions were:

Medium:	Water
Temperature:	25°C
Liquid Volume:	100% working bag capacity

Iodometry decolourisation

Cameras were set-up for both front facing and side angles of the bioreactor. To show even colourisation of the liquid, 4mL/L of Lugol's iodine solution and 5mL/L starch solution were added to the bioreactor bag filled with the medium. The solution was allowed to mix for 5 minutes to ensure complete homogeneity and to establish a regular flow pattern.

Once the liquid flow pattern has stabilised, 4mL/L sodium thiosulphate solution (the decolourisation agent) was added and the time to complete decolourisation was measured. The timer was stopped when colour change from dark blue to colourless was achieved throughout the bioreactor bag.

In order to objectively determine and scrutinise full decolourisation of the liquid, we utilised video recording to determine exact time points of test completion. Representative recordings of the procedures in this study are available on-line (https://youtu.be/L0Sa7oeb2uQ).

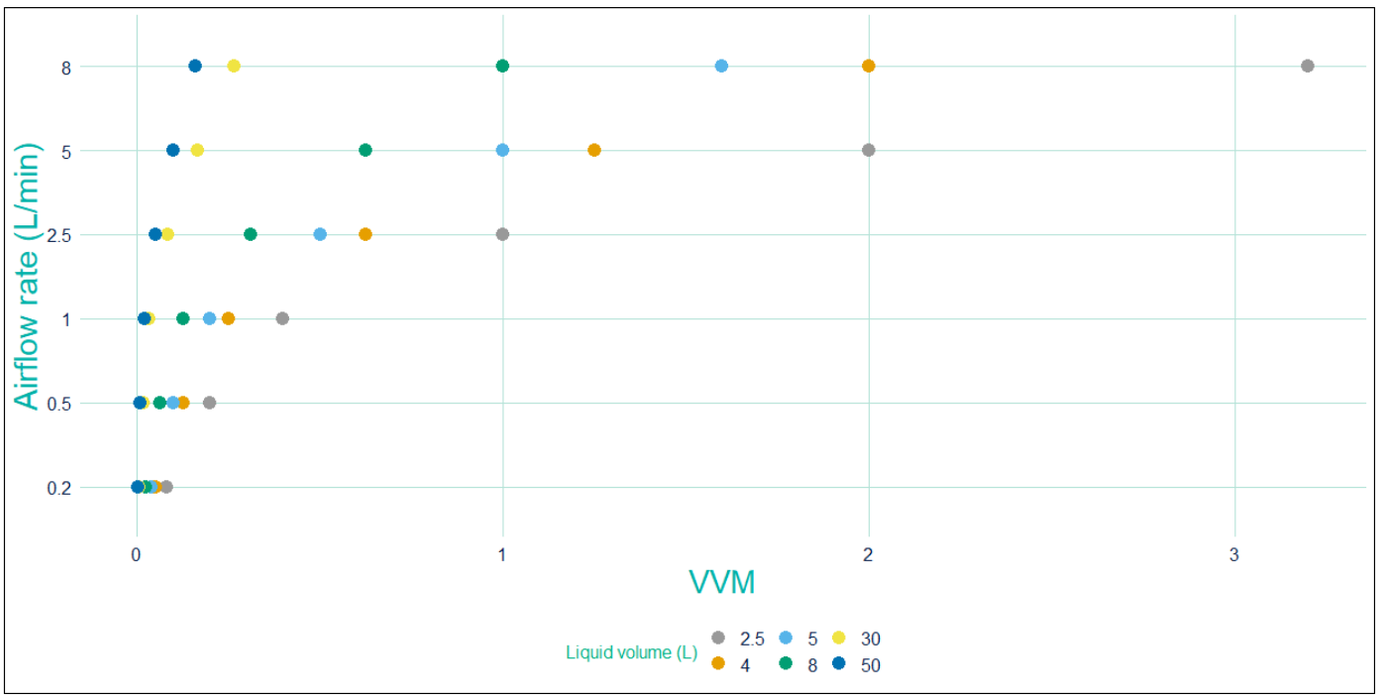
Volumetric mass transfer coefficient

Experimental conditions

A series of experiments was performed in the CellMaker Plus 4L, 8L and 50L single-use bioreactor systems for a varied levels of air input and media volumes. The experimental conditions were as follows:

Medium:	PBS Solution in deionised water
Temperature:	37°C
Purge Gas:	Nitrogen
pH:	7
Liquid volume:	65% of maximum bag working capacity

Compressed air was used to supply atmospheric air to avoid the pulsating effect casued by the built-in air pump on the airflow. This ensured immediate target flowrates were achieved subsequent to the nitrogen purging without any delay of the internal air pump ramping to the desired flowrate.



Airflow \ Volume	2.5	4	5	8	30	50
0.2	0.08	0.05	0.04	0.025	0.00666	0.004
0.5	0.2	0.125	0.1	0.062	0.0166	0.01
1.0	0.4	0.25	0.2	0.125	0.0333	0.02
2.5	1.0	0.625	0.5	0.312	0.0833	0.05
5.0	2.0	1.25	1.0	0.625	0.166	0.1
8.0	3.2	2.0	1.6	1.0	0.266	0.16

Figure 1. Calculation of the VVM parameter for fermentation liquid volumes tested. Tested volumes are represented by individual colours in the graph. Precise values of each represented point are presented in the data table.

k_La determination

The k_La measurements were conducted based on the SOP published by DECHEMA with modifications (DECHEMA, 2016, ISBN 9783897461710). 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 4L, 8L and 50L models filled with 2.5L, 5L and 30L of PBS, respectively.

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

$$(1)\ \ln\left(DO^*_{max} - DO(t)_{exp}\right)$$

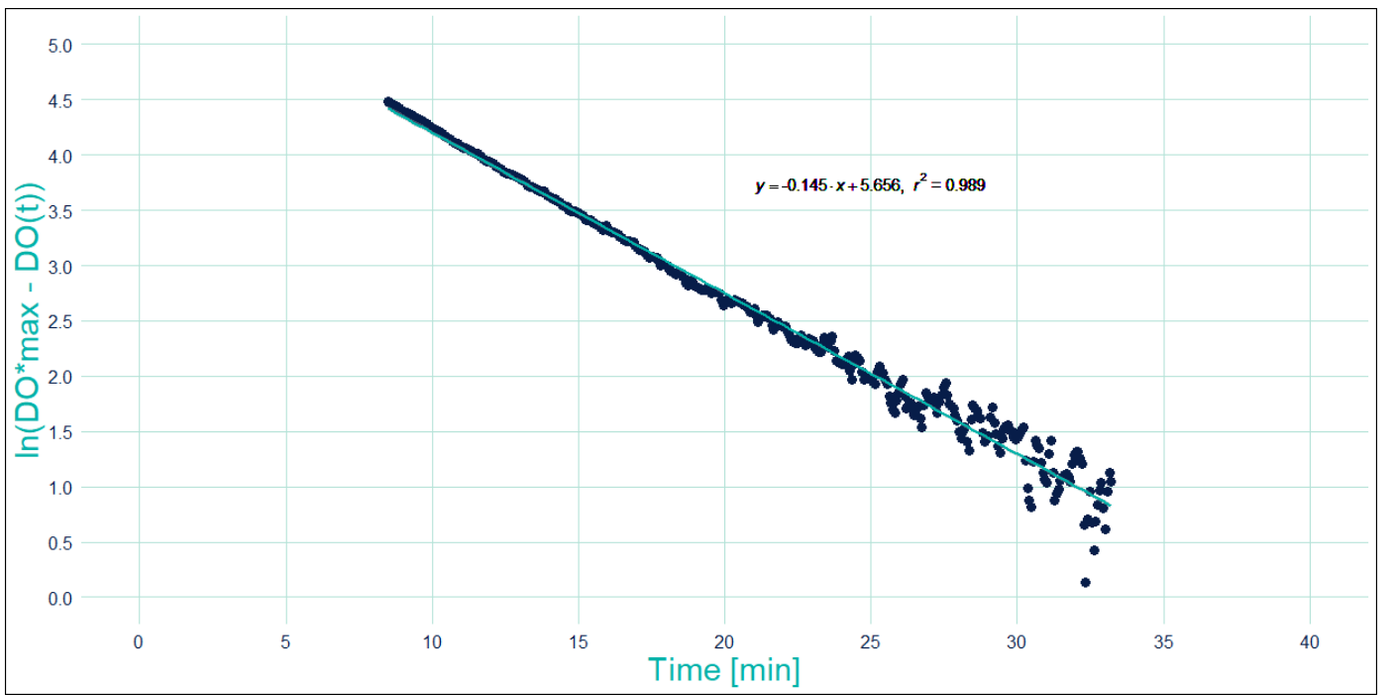


Figure 2. Determination of the k_La value in range of 10 to 90% of max dissolved oxygen ($\%DO_{2max}$) in the CellMaker 8L bioreactor bag using airflow of 0.2L/min.

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 2.

$$(2)\quad k_La\left(0.2\ L\ min^{-1}\right) = \frac{1}{n}\sum_{i=1}^{n} k_La_i = \frac{8.688\ hr^{-1} + 11.844\ hr^{-1} + 8.718\ hr^{-1}}{3} = 9.75\ hr^{-1}$$

Results

The CellMaker provides even and efficient mixing

A CellMaker system equipped with an 8L Enclosure provides a wide range of working fermentation volumes (from 1.5L to 8L working volume) and is the most common choice among the CellMaker users. Thanks to its flexibility, this system is ideal for optimisation and pilot scale fermentation stages. The CellMaker can supply atmospheric air using an integrated air pump at the rates from 0.5 to 10 L/min, depending on the model. To investigate the effectiveness of liquid mixing in a CellMaker system at various scales, we used the VVM parameter to assess the performance of the system. In the 8L system, we assessed media mixing at VVM from 0.1 to 2.5 (Figure 1).

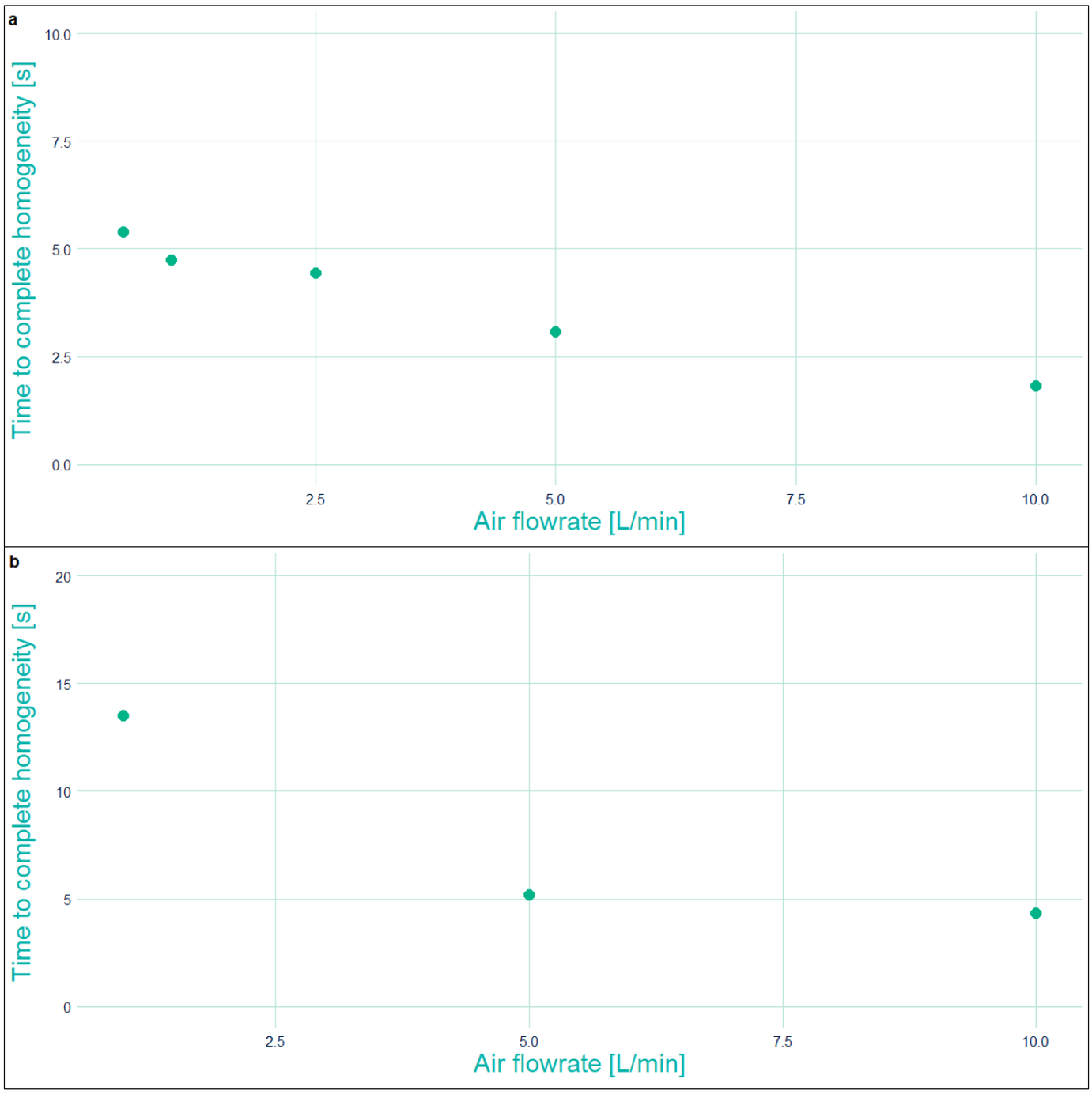


Figure 3. Time required for complete decolourisation of Lugol's iodine-starch solution in a CellMaker 8L system fitted with a) a 4L Bioreactor bag and b) an 8L Bioreactor bag at air flowrates of 0.5, 1, 5 and 10L/min.

Figure 3 shows the relationship between mixing efficiency and air flowrates in a 4L bag (3a) and an 8L bag (3b). As expected, the time to complete mixing of the medium was the longest at the lowest VVM of 0.125 in both bag models. Increasing the flowrate to achieve the VVM of 0.625 (2.5L/min in a 4L bag and 5L/min in the 8L bag) has more than halved the time required to mix the medium completely. There was little difference between mixing efficiency at 5 and 10L/min, VVM of 2.5 and 1.25, respectively.

At the maximum fill of the 8L bioreactor bag, 5 seconds is the shortest time necessary to completely mix the media. Importantly, the distribution of the colour within the bag, during the discolouring phase of the experiment, has not indicated any dead spaces within the bioreactor. This demonstrates the efficiency of mixing provided by the patented shape of the CellMaker bioreactor bag.

The maximum working volume of 50L is ideal for a medium scale bioproduction processes. Such work will frequently involves additives or feeds during the fermentation, or an advanced intervention like perfusion, during a continuous batch fermentation. It is essential to ensure the contents of the bioreactor chamber is efficiently, and thoroughly, mixed with no unmixed dead spaces occuring. At the increased volumes, the VVM parameter is generally lower in a 50L than in an 8L system. Thus, we expected the mixing times to be longer and we tested air flowrates of 1, 3, 5 and 10L/min (Figure 1). Figure 4 and Table 1 show the relationship between mixing efficiency and air flowrates. The mixing times ranged from 32s at the flowrate of 1L/min to 11s at 10L/min. Once again, the pattern of decolourisation has confirmed no dead spaces forming in the bioreactor bag, even at such a high volume.

Volume / Airflow	4	8	50
0.5	5.35	N/A	N/A
1	4.55	13.5	32.17
2.5	4.12	N/A	21.00
5	3.15	5.17	14.25
10	1.82	4.33	10.67

N/A - Not assessed

Table 1. Time (in seconds) to complete decolourisation of Lugol's iodine-starch solution in Cellexus CellMaker 4, 8 and 50L bioreactor bags.

The mixing times in the 50L bag are longer than in the 8L bag, as expected. The VVM at 1L/min in an 8L system is 0.125, which resulted in complete mixing time of 13.5s. Comparably, to achieve the same VVM in a 50L system, the required air flowrate is 6.25L/min. At the nearest tested flowrate of 5L/min, we achieved complete mixing of the bioreactor liquid in 14.25s. These results show, that mixing time in the CellMaker bioreactors is proportional to the VVM factor, as at 1L/min in the 8L bag (VVM of 0.125) and 5L/min in a 50L bag (VVM of 0.1) the mixing times are similar and continue at the same trajectory in all bag models (compare Figure 3 and Figure 4).

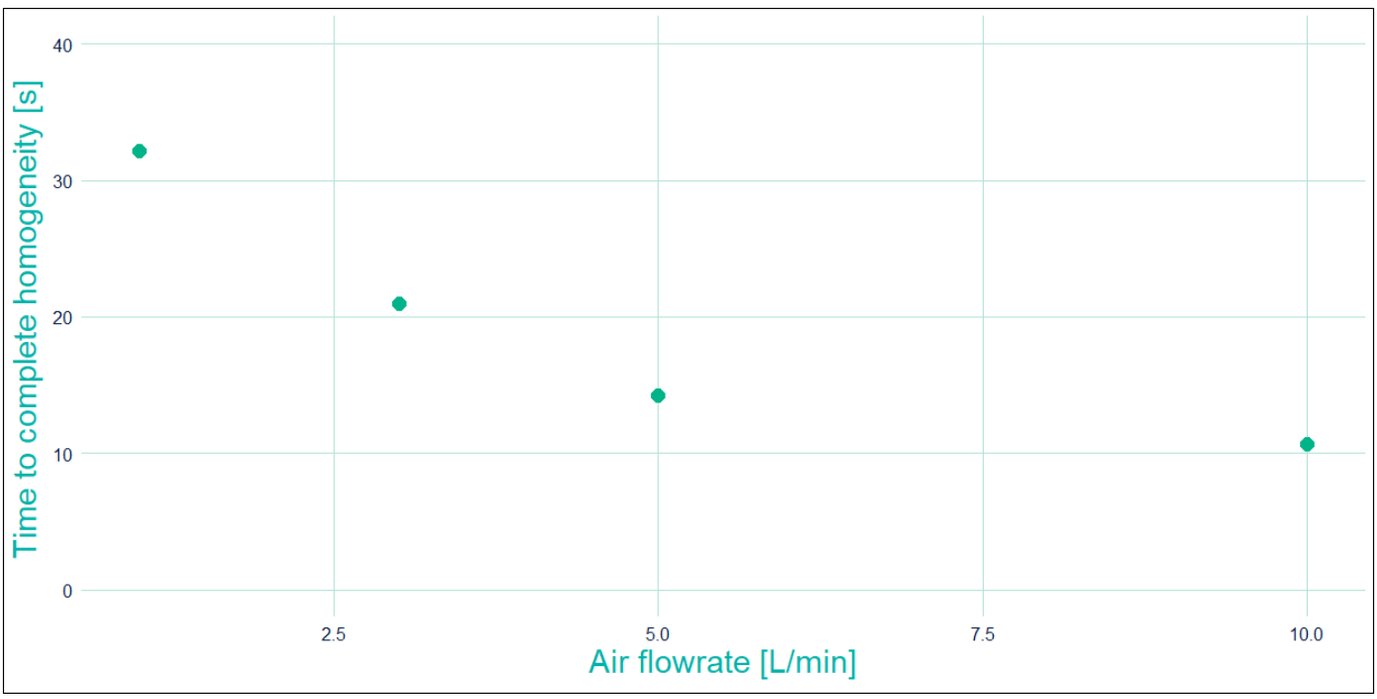


Figure 4. Time required for complete decolourisation of Lugol's iodine-starch solution in a CellMaker 50L system with air flowrates of 1, 3, 5 and 10L/min.

Airlift aeration is an efficient method of gas distribution

The most commonly used type of a bioreactor is the continuously stirred tank fitted with a rotating impeller to disperse the gas injected by a vertical microsparger. The microsparger is typically located directly underneath the impeller's blades, and the released gas bubbles are disrupted by the motion of the blades.

The CellMaker system is free of mechanical parts in the bioreactor bag. The movement of the fermentate and aeration are entirely provided through the airlift process: Gas is injected into the fermentation liquid through a horizontal sparger spanning the whole width of the bag. The rising gas mixes the liquid and dissolves in it as the gas bubble travels upwards. The pore size of the sparger was selected to provide efficient mass exchange rates while maintaining low shear energy delivered to the cells. 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 fermentations 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 3:

$$(3)\quad OTR = k_L a\,(\%DO_{2max} - \%DO_2)$$

This contains the volumetric mass transfer coefficient (k_La) as the driving parameter which can be separated 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_La. We have measured the k_La values in the CellMaker Plus system, which allows precise control of air flows while measuring the level of dissolved oxygen ($\%DO_2$) in the media among other parameters. To reflect on the scalability provided by the CellMaker systems, we used the VVM parameter (Figure 1) as the means to compare the efficiency of mass transfer at different fermentation scales. In this analysis, we chose to use the bag fill volume most reported by the CellMaker systems users, which is 65% of full capacity. This is 2.5L for the 4L bag, 5L for the 8L bag and 30L for the 50L bag, respectively. The k_La was measured during linear $\%DO_2$ increase, that is between 10% and 90% of $\%DO_{2max}$.

The CellMaker 4L bioreactor bag is the smallest of the bags in the CellMaker range, which we predicted to produce the highest k_La values. The bag was filled to 2.5L with PBS and oxygen was purged by sparging the media with nitrogen. Once dissolved oxygen sensor reading stabilised at 0%, we supplied compressed air at various air flows from 0.26L/min (VVM of 0.065) up to 5L/min (VVM of 2) (Figure 5a). Even at the lowest flow rates tested, the k_La value in the 4L CellMaker bag was on average 26.2 (±0.46) reaching 298 (±25) at 5L/min (Table 2).

Flow rate (L min^{-1})	0.26	0.5	1	2.5	5
kLa value ($hour^{-1}$)	28.20 (±0.46)	42.71 (±0.83)	80.20 (±5.04)	182.03 (±4.74)	298.56(±25.03)
VVM	0.1	0.2	0.4	1	2

Table 2. k_La values in the CellMaker 4L bioreactor bag sparged at 0.26 – 5L/min of compressed air.

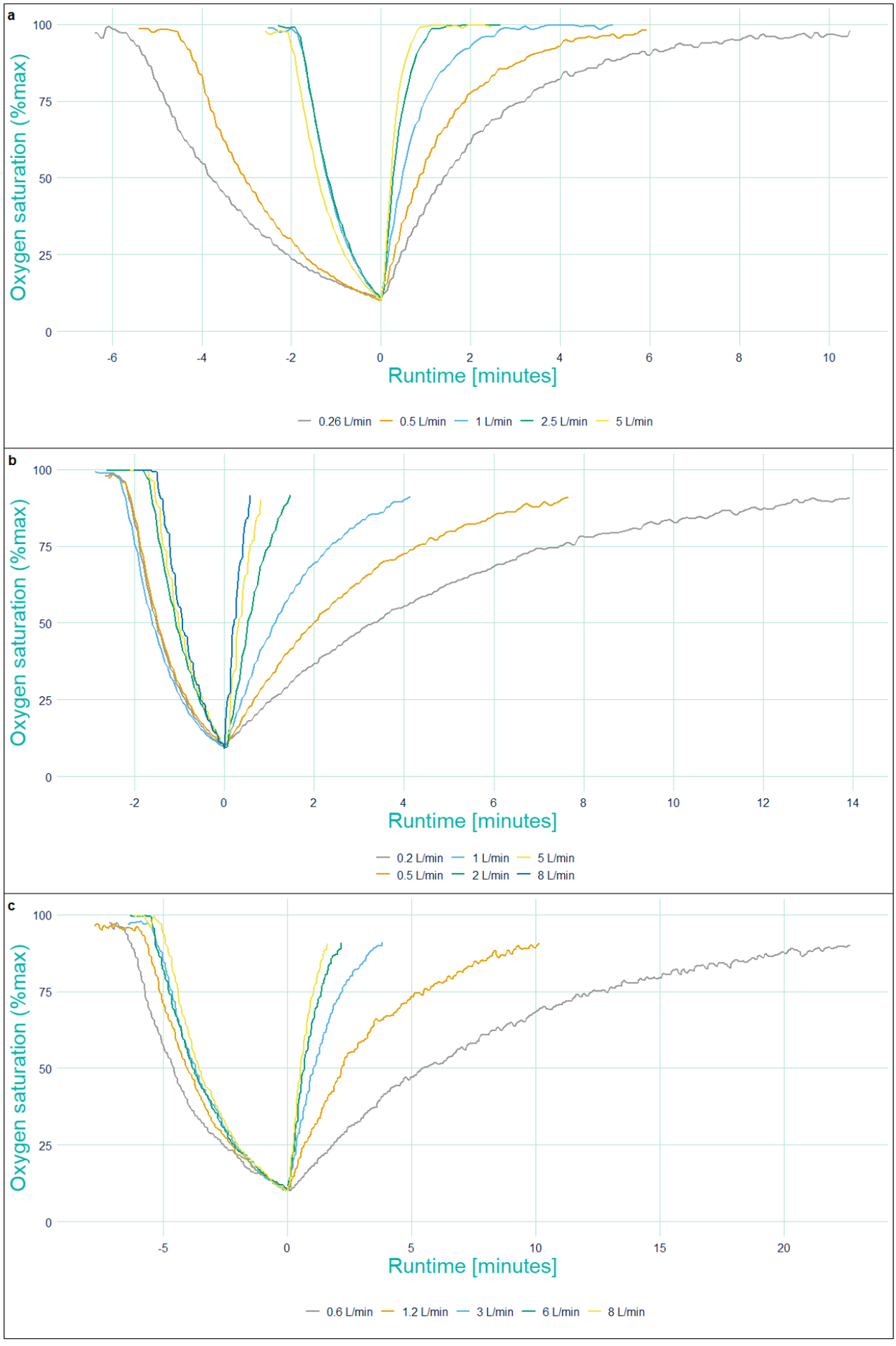
The 8L bioreactor bag, due to its ideal placement in the middle of the working volumes range, allowed us test the full spectrum of the air flowrates available from the controller. Using the VVM parameter as the basis of scale comparability, the 4L and 8L Bioreactor bags have displayed comparable results (Table 3, Figure 5b). At the maximum flowrates possible to reach within the pressure limits of the system, i.e. VVM approx. 2, the k_La in both bags was above $220h^{-1}$ using atmospheric air only.

Flow rate (L min^{-1})	0.2	0.5	1	2.5	5	8
kLa value ($hour^{-1}$)	8.40 (±0.47)	20.98 (±1.17)	35.7 (±1.50)	102.06 (±1.75)	157.83 (±4.59)	224.26 (±3.09)
VVM	0.04	0.1	0.2	0.5	1	1.8

Table 3. k_La values in the CellMaker Plus 8L system sparged at 0.2 – 8L/min of compressed air.

The measurements of the k_La values were repeated using the 50L CellMaker bioreactor bag filled with 30L of media. This is a corresponding level of fill to the 5L of media in an 8L bioreactor bag. The CellMaker Controller delivers the same airflow rates to all systems, regardless of the connected Enclosure, thus the VVM values we obtained were respectfully lower (Table 4 and Figure 5c).

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


a
Oxygen saturation (%max)
100
75
50
25
0
-6
-4
-2
0
2
4
6
8
10
Runtime [minutes]
0.26 L/min
0.5 L/min
1 L/min
2.5 L/min
5 L/min
b
Oxygen saturation (%max)
100
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14
Runtime [minutes]
0.2 L/min
0.5 L/min
1 L/min
2 L/min
5 L/min
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c
Oxygen saturation (%max)
100
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-5
0
5
10
15
20
Runtime [minutes]
0.6 L/min
1.2 L/min
3 L/min
6 L/min
8 L/min

Figure 5 (previous page). A representative graph of k_La measurement by degassing method in a) 2.5L of media in a 4L CellMaker bioreactor bag, b) 5L of media in an 8L bioreactor bag, and c) 30L of media in a 50L 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 airflow 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.

Flow rate (L min^{-1})	0.6	1.2	3	6	8
k_La value ($hour^{-1}$)	5.33 (±0.79)	14.13 (±0.52)	39.07 (±1.32)	66.02 (±1.82)	84.15 (±0.34)
VVM	0.02	0.04	0.1	0.2	0.27

Table 4. k_La values in the CellMaker Plus 50L system sparged at 0.6 – 8L/min of compressed air.

Asymetrical geometry of the bioreactor bag improves gas exchange at increasing volumes

One of the key properties of the CellMaker systems is the flexibility of the fermentation media volume, 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. The unique asymmetrical shape of the CellMaker bag has a big influence on how the fermentation liquid is moved in the bioreactor bag and on the mass transfer kinetics. Therefore, we investigated the relationship between the volume of media with respect to gas flowrate. For this purpose, we measured the k_La values at a constant air flowrate of 1L/min at increasing media volume, thus modulating the VVM parameter (Table 5).

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

Table 5. k_La values for aeration of 3, 5 or 8L of media at 1L/min airflow in the CellMaker Plus 8L system.

As expected, the k_La values dropped with the reduced VVM. The step-change of VVM from the 3L of media to 8L of media is 2.66-fold however, the k_La has reduced only 1.88-fold. We attribute this non-linear relationship to the specific geometry of the bioreactor bag, which allows for much more efficient gas mass exchange, than achievable in a symmetrical container.

CellMaker aeration efficiency compared to industry leaders

The specific oxygen transfer coefficient (k_La) values are essential in any bioreactor system, and one of the key parameters impacting the performance. 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), the bioreactor system needs to provide enough oxygen to fuel the bioprocess.

The conventional stirred tank bioreactors are characterised by the most efficient k_La values reaching even $600h^{-1}$, 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 and affect the stability of the produced molecules. On the other hand 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 very low, typically in range of 1 to $15h^{-1}$.

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. Our data indicate that the CellMaker airlift bioreactor can deliver the k_La values in ranges comparable to most other systems on the market.

With the low airflow 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 show that the oxygen transfer at these airflow 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 and well within the requirements of the CHO cell line (approx. $8h^{-1}$) (Figure 6).

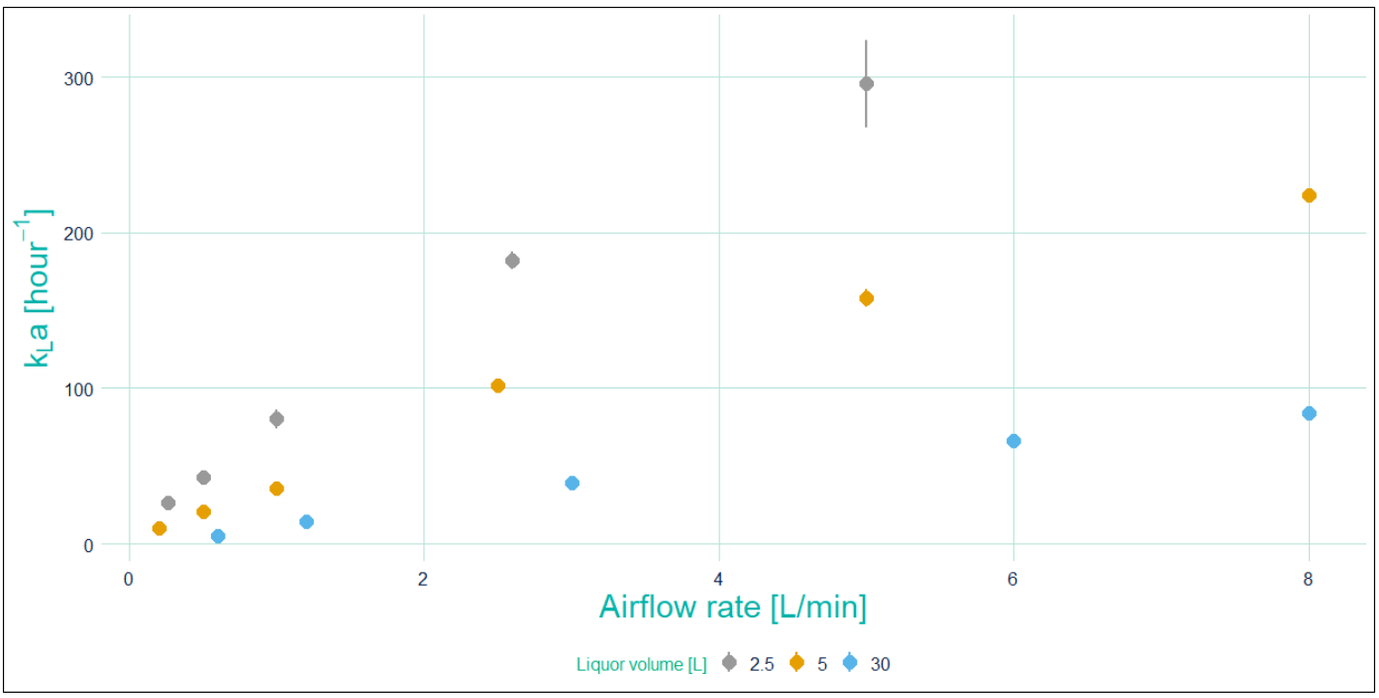

Figure 6. The k_La values achieved by the CellMaker system filled with 2.5L (grey points), 5L (orange points), or 30L (blue points) of media and sparged with air at indicated rates. Each point represents an average from 3 independent measurements and the whiskers are standard deviation.

The second point of concern was the k_La value at high airflows (5 – 9L/min) required for high cell density microbial cultures. Indeed, the k_La values of the CellMaker are not as high as 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 airflows (VVM approx. 1.8), the CellMaker can deliver k_La exceeding 200h^{-1} (Figure 6), which is comparable to standard stirred tank single use system.

Concluding remarks

Maintaining culture homogeneity, an even distribution of nutrients, gases and cells, in large-volume cultures is the main reason for choosing to work with bioreactors. Over the decades of technology development, a plethora of bioreactor designs have been produced, each with its own benefits. Here we described the capacity for efficient liquid mixing and mass transfer of gases of a unique, airlift bioreactor, the CellMaker. Operating 3 different sizes of single-use bioreactor bags, the CellMaker offers options of process development from pilot to production scale. The CellMaker bioreactors have been designed with a specific geometry to optimise mixing and aeration in the bioreactor bag.

The data presented in this report show that even at the maximum capacity of the 50L bioreactor complete mixing of the fermentation liquid can be achieved in approx. 30s. By decreasing the VVM value, this result can be improved to just a few seconds (Table 2).

The results 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 is only beaten by the stirred tank systems with the highest k_La values.

The CellMaker is very easy to assemble and operate, even at 50L. This single-use system allows for smooth change of bioprocesses without the risk of cross-contaminating individual fermentation batches. 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 caused by 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 supplementary 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 in this study. The mixing times and the high k_La results presented in Figure 7 show inverted trends with the steepest slope up to the VVM of 0.25 and then gradually levelling off. This shows a direct relationship between air flowrate in the CellMaker systems and both mixing efficiency as well as aeration of the fermentation medium, confirming the CellMaker as a highly efficient, balanced fermentation system with low shear stress..

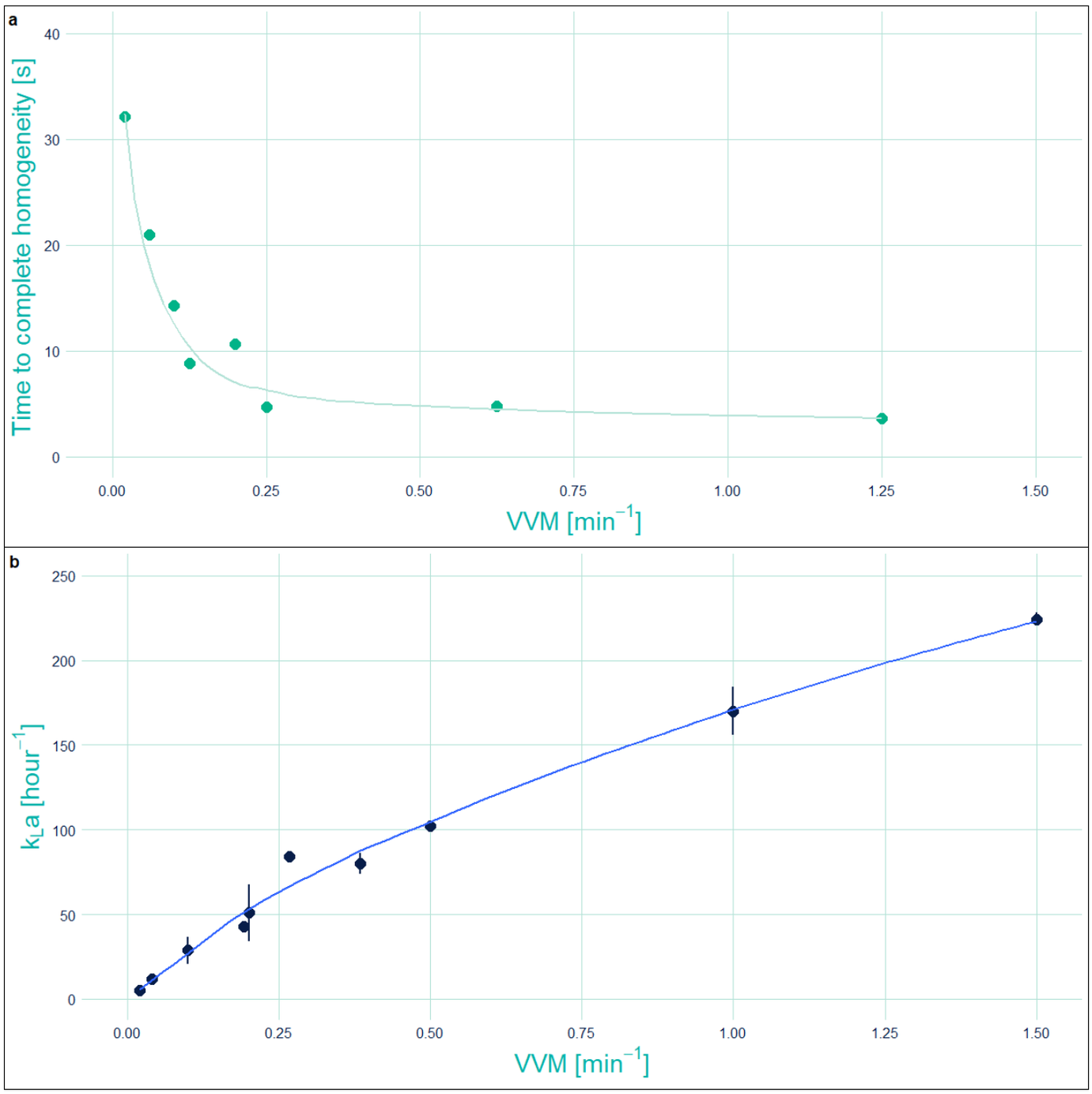

Figure 7. a) The time required to achieve complete mixing of the fermentation medium, and b) the mass transfer coefficient k_La in relationship to the VVM parameter in the CellMaker single-use bioreactor systems. The graphs represent combined results obtained from the measurements in the 4L, 8L and 50L bioreactor bags. Solid lines represent best fit curves.

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