Technical Application Note: Utilization of a Novel k_La Measurement Procedure for Bioreactor Characterization and Optimization to Assist with Compliance of Regulatory Guidelines

Background Information and Introduction

CGMP regulations stipulate that sampling and testing of in-process materials and drug products requires control procedures to "be established to monitor the output and to validate the performance of those manufacturing processes that may be responsible for causing variability in the characteristics of in-process material and the drug product". [(1)]

According to USFDA guidelines, utilizing validated processes is critical to ensuring a drug is fit for its intended use. This guidance describes process validation activities in three stages:

- Stage 1 – Process Design: The commercial manufacturing process is defined during this stage based on knowledge gained through development and scale-up activities.
- Stage 2 – Process Qualification: During this stage, the process design is evaluated to determine if the process is capable of reproducible commercial manufacturing.
- Stage 3 – Continued Process Verification: Ongoing assurance is gained during routine production that the process remains in a state of control.

Information and data should demonstrate that the commercial manufacturing process is capable of consistently producing acceptable quality products within commercial manufacturing conditions.

Manufacturers should:

- Understand the sources of variation.
- Detect the presence and degree of variation.
- Understand the impact of variation on the process and ultimately on product attributes.
- Control the variation in a manner commensurate with the risk it represents to the process and product. [(2)]

The FDA's Biotechnology Inspection Guide (11/91) states that "it is important for a bioreactor system to be closely monitored and tightly controlled to achieve the proper and efficient expression of the desired product. The parameters for the fermentation process must be specified and monitored." [(3)]

The bioreactor is at the heart of any cell culture process in which microbial, mammalian, insect or plant cells are used to manufacture biological drug products. The main function of a bioreactor is to provide a controlled environment to achieve optimal growth and/or product formation in the particular cell system employed [(4)]. However, by definition, being a biological system – albeit designed to be controlled – a bioreactor culture is subject to the same variations that exist in any other biological system in nature.

In such a context, where natural variations can occur, it is critical to ensure optimal bioreactor performance based on a validated process design. Guaranteeing reproducible, steady-state conditions requires accurate measurement and control of factors that influence the process. These factors include a number of chemical and physical parameters, including culture media type and components, pH, aeration, agitation, temperature and culture time. [(5)]

The Importance of k_La

It has long been established that, as a function of aeration and agitation, one of the most pertinent performance parameters to measure consistently is the volumetric mass transfer coefficient (k_La). It describes the efficiency of gas (oxygen) transfer from the gaseous to liquid phase and is often used to monitor the efficacy of bioreactors and as an important factor in scale-up operations. Dissolved oxygen (DO) is a limiting factor in bioreactors. Therefore, for optimum cell growth, it is important that DO concentrations are maintained - throughout the whole bioreactor - at or above the critical oxygen thresholds for the cells being cultured. To be effective, the oxygen transfer rate (OTR) - following its gaseous introduction into the bioreactor, via the sparger, into the liquid phase of the culture medium - must equal or exceed the oxygen uptake rate (OUR) of the cells in the vessel. The OTR, and the kLa in particular, are critical parameters in the design of bioreactors. [6] [7]

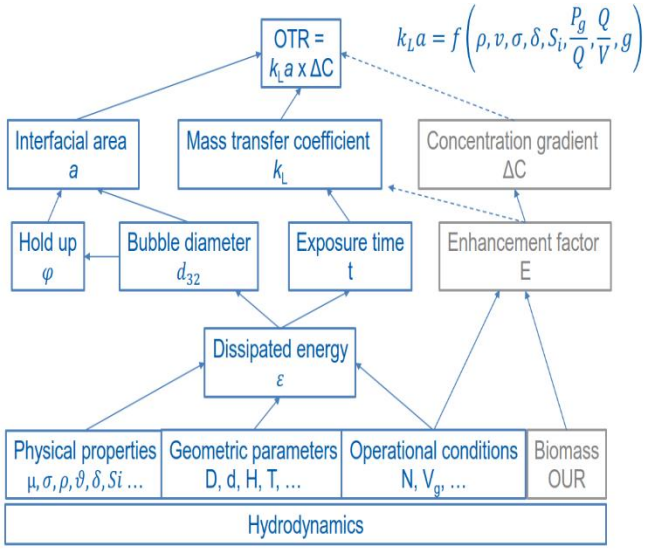


Fig. 1 – Correlation between OTR, the kLa coefficient and hydrodynamic parameters in a bioreactor. [8]

The k_La and OTR values are influenced by a number of factors. They include process parameters (agitator speed, temperature, pH, and gassing rate), physiochemical characteristics of the culture medium (viscosity, density, salt content, surface tension, and coalescence behavior) and equipment geometries (vessel, agitator, and sparger).

Therefore, given the number of variables that can affect the k_La, and the regulatory authorities' demands for manufacturing processes to be tightly monitored and controlled, it places even greater emphasis on being able to characterize bioreactors by accurately measuring this coefficient. Currently, there are numerous ways of measuring the k_La utilizing chemical or steady-state and physical or dynamic methods.

Standard Methodology for Measuring k_La

Typically, two approaches are adopted for measuring the k_La:

1. Dynamic - These methods consist of producing an oxygen concentration change in the entering gas, recording the dissolved oxygen change in the liquid and fitting the transient curve to a proper model:
    a. The “gassing-in” method (GIM) consists of a step-change in the gas while keeping the gas flow rate and agitation unchanged.
    b. the “gassing-out” method (GOM) utilizes the opposite interchange.
    c. The “dynamic start-up method” (DSM) consists of starting agitation and simultaneously feeding air or pure oxygen to the liquid deoxygenated by stripping with nitrogen, (Linek et al., 1987a).
    d. The “dynamic pressure method” (DPM) relies upon inducing a concentration variation in the liquid by step-wise changing the pressure in the system (Linek et al., 1989).
2. Steady-state methods – These methods rely upon a chemical or a biochemical reaction taking place in the liquid, which acts as an oxygen sink. A number of variants have been used that depend on the specific reaction:
    a. Sodium sulphite oxidation method
    b. Carbon dioxide absorption method
    c. Hydrazine oxidation method
    d. Catechol bio-oxidation method
    e. Glucose oxidase method
    f. Krypton absorption method

More details of these techniques, and the relative merits of each, can be found elsewhere in the scientific literature and will not be discussed further here.

Fig.1 illustrates that the k_La value depends on a number of hydrodynamic conditions, therefore, it cannot be predicted precisely. Mathematical models describing the system kinetics represent an important base for prediction of k_La values. Oxygen sensors typically are used for determination of the actual k_La in a culture, and until recently, such measurements took place at only one point in a bioreactor - at the probe belt near the bottom of the vessel – thus introducing a greater risk of increased and undetected process variation, a reduced state of control and reproducibility, monitoring inaccuracies and sub-optimal performance. In practice, DO varies greatly in its distribution throughout the bioreactor, so a single measurement is not representative for the whole vessel and does not take into account any “zoning” effects that may occur.

New Methodology for Measuring k_La

This application note details a new and novel technique, utilizing a dynamic start-up method (DSM), outlined above, to accurately determine the coefficient, which is then extrapolated into characterizing the whole bioreactor, thus making it easier to comply with the regulatory requirements. A number of case studies where the technique has been applied will also be discussed. Strategies employed to improve the accuracy of k_La measurement for bioreactor characterization are as follows:

DSM Methodology – Under experimental conditions, the bioreactor was filled with deionized water. The water was then degassed with nitrogen until the oxygen concentration was constant and below a value of 0.1% saturation. Then the gassing was immediately switched to air, at a pre-determined rate, to increase the concentration of oxygen to 100% air saturation, before the k_La values were measured.

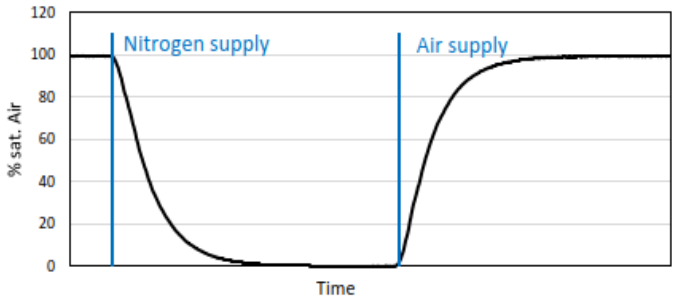


Fig.2. - Schematic illustration of the concentration change when using the DSM.

Flow-Through Cell - This method, incorporating the use of a flow-through cell oxygen sensor, strips all gas bubbles from the sample allowing the sensor to read a more accurate noise-free signal (see patent number A20182/2018). The in-line sensor has been developed to be compatible with any sampling port on a bioreactor. As the measuring method positions the sensor outside of the vessel, with the sample being drawn through tubing inside the bioreactor, it allows for any part of the vessel to be surveyed (See Fig.3.). A peristaltic pump – run at a pre-calibrated speed - guides sample material to an optical high-speed sensor that measures DO within 0.25 seconds.

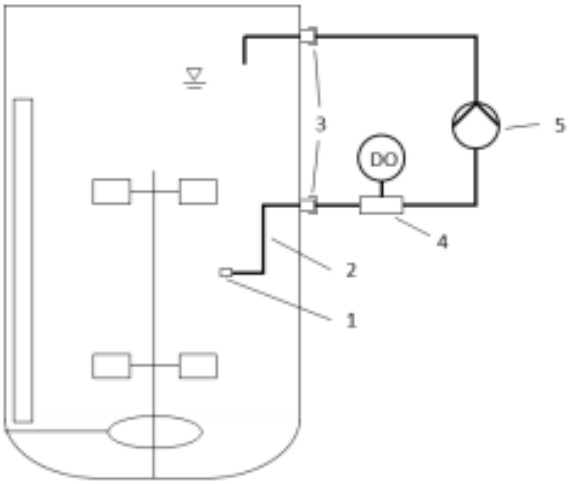


Fig.3. - Schematic illustration of the measuring method. (1) membrane and frit (2) sample inlet pipeline (3) Connector to the reactor (nozzle). (4) flow-through cell (5) peristaltic pump for sample return.

Fig.4. illustrates that there is no significant difference in the calculation of k_La when using the bubble-stripping flow-through cell method compared to the traditional standard sensor. This allows for the DO distribution of the whole of the bioreactor to be reproducibly and repeatedly mapped, giving full visibility of any zoning or areas of oxygen under-supply, for any given set of process parameters. Research on varying process parameters or bioreactor design can then be carried out to investigate the removal or reduction of these zones.

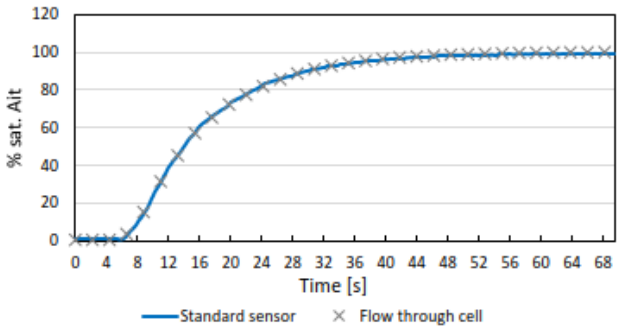


Fig.4. – Oxygen concentration curves comparing the standard method with that of the flow-through cell.

Gas Bubble Stripping - A gassed bioreactor represents a two phase system with a continuous liquid phase and a dispersed gaseous phase. Since only the DO concentration of the liquid is of interest, the presence of gas bubbles has a negative influence on the overall measurement. Furthermore, gas bubbles coming into contact with an oxygen sensor membrane can generate signal "noise" and cause further reading inaccuracy. To investigate the influence of the two phase system, measurements were taken, with the standard probe and the flow-through cell, for comparison (see Fig.5.). It can be seen with the standard sensor that there is considerable signal "noise", giving rise to more variability and inaccuracy, particularly in the early ramp-up phase. This is not observed in the flow-through cell trace where bubble-stripping occurs.

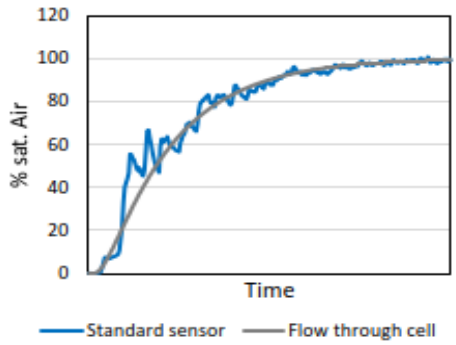


Fig.5. Comparison of sensor readings when comparing the standard and flow-through cell methods.

Case Studies Utilizing New Methodology for Measuring k_La

1. Optimization of Existing Production Scale Bioreactors

As this newly developed measuring procedure is also mobile it can be applied to bioreactor systems already in existence without the need for structural changes. In this application, a 12,000L working volume, commercial scale bioreactor – used for recombinant protein production in *E. coli* - required characterization. For the analysis, the previously described DSM method was employed in three defined areas of the vessel: at the top, in the middle and at the bottom. Across 20 trials, measurements were taken to explore the effects of different agitator speeds, gassing rates, head pressures and salt and anti-foam levels in the fermentation medium. Fig.6. shows the positioning of the oxygen sensors within the bioreactor.

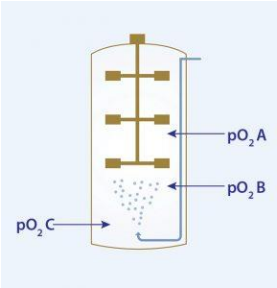


Fig.6. k_La measurement points within 12,000L bioreactor (type and position of sparger and impeller also shown).

By measuring k_La at multiple points, it was clearly identified that zoning was occurring. The data gathered showed that the central area of the bioreactor exhibited the highest k_La value and was providing the most favourable conditions for cell growth. Fig.7. shows the spatial difference in measured k_La, for a 12,000L bioreactor, and how it increases linearly with increased gassing rates, at a fixed agitation rate.

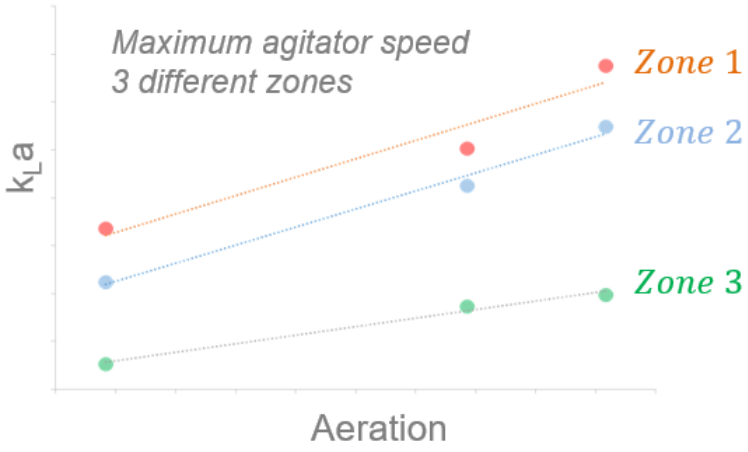


Fig.7. Spatial differences in the correlation of measured k_La with increased aeration and constant agitation speed.

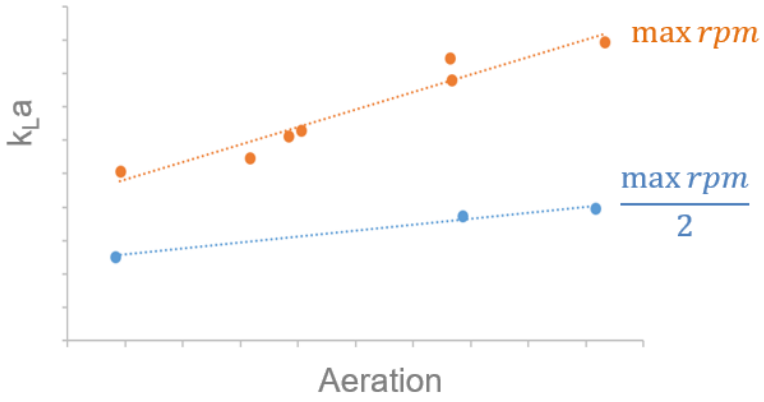


Fig.8. Influence of Power Input (agitator speed [rpm]) on the k_La value with increased aeration measured at the "sweet spot" of the fermenter

The oxygen dissolution from the head space has a significant impact on the k_La value within this zone (sensor pO2A) The study showed that at higher agitator speeds – due to improved dispersal of gas bubbles - the zoning effect was reduced and an optimum gassing rate above 1.5 vvm was recorded. It was shown that a slow agitator speed was the greatest limiting factor to achieving an optimal k_La value.

Being able to determine the spatial differences in k_La observed in this bioreactor, it provides invaluable assistance in optimally installing other vessel fittings. For example, both the carbon source feed rate and feed-pipe positions are important for product yield and quality. In this instance, the end user was able to add an additional submerse feed tube at an optimal bioreactor position, with the higher DO concentration, and achieve a greater productivity.

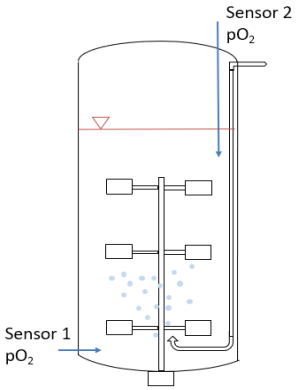
In conclusion, the new method for k_La determination enabled the collection of more representative empirical data, that provided quantitative insights into the bioreactor dynamics. This facilitated the enhancement of an existing manufacturing process through the reduction of variation and the improvement of control and product quality attributes. Moreover, these empirical characterisation data, like k_La and mixing time, were used to setup a scale-down model which guarantees new drug and strain developments in lab and pilot scale fermenters mimicking the commercial scale characteristics in detail. By operating an exact twin in smaller scale reduces the risk of scale up failures extremely and saves time by possibly reducing the amount of engineering and validation runs.

2. Characterization of Production Scale Bioreactor

This application required a 200L fermenter - used in a commercial process producing recombinant protein (Insulin) in *E. coli* - to be characterized to allow the end user to make informed decisions on the best operational process parameters to use.

Again, the previously described DSM method was employed in two defined areas of the vessel: close to the top and at the bottom (see Fig.8.). At a fixed temperature of 30°C, a working volume of 200L and an ambient head-space pressure, more than 20 trials (with replicates) were conducted across a range of agitator speeds and gassing rates.

Fig.9. Oxygen sensor locations within 200L bioreactor.



For bacterial high cell fermentations the k_La value can influence the growth rate and finally the biomass concentration directly. The goal is to reach highest k_La values and reducing the “zoning” effect at the same time. “Zoning” accures when the impeller design is choosen for optimal mass transfer and highest power input. The drawback of radial flow agitators is the lack of forming strong bulk mixing flow regimes. The result is the formation of vertical zones with poor zone intermixing capacity. This fermentation system is tuned for highest mass transfer rates using a magnetic agitator equipped with 3 stage hollow blade impellers. After defining the design space with the end-user (Figure 10) the fermenter characterisation experiments were carried out. Empirical results of this phenomenon are shown in Figure 11.

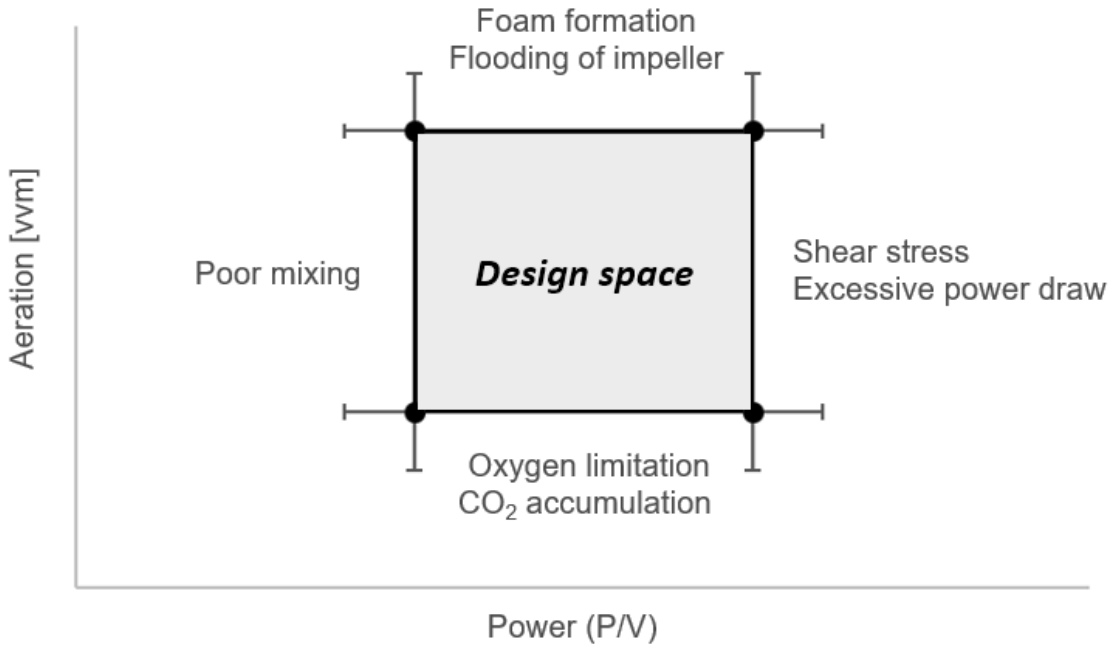


Fig.10. Defining the design space for the bioreactor characterisation

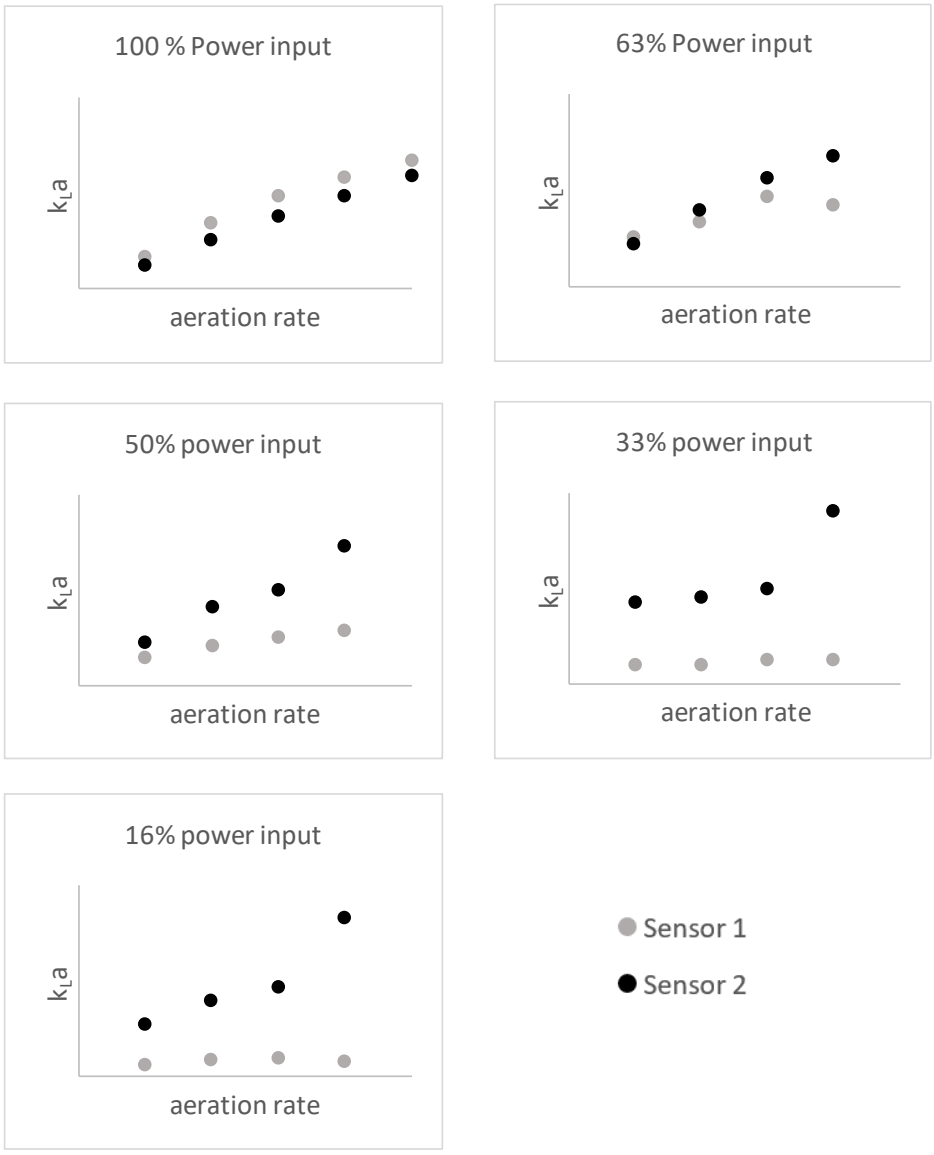


Fig.11. Experimental results showing different k_La values measured across a range of gassing rates and agitator speeds.

At highest energy dissipation rates no zoning occures irrespective of aeration rates – a equal k_La distribution is formed. The power input of the agitator is high enough to guarantee an efficient gas phase distribution without the risk of flodding. At lower agitator speeds "zoning" occures as there is a significant difference in k_La between the sensor at the top (high) and at the bottom (low) of the stirred tank. At lower power input and high gassing rates flooding of the lowest impeller is observed. The lowest hollow blade impeller was not able to disperse the high air flow, is partly flooded and all the air bubbles start breaking up at the 2nd and 3rd stage of the agitator resulting in very high k_La values in the top area of the fermenter.

In conclusion, critical process parameter limits were defined. The detailed insight into the fermenter performance provides a scientific basis for further product and process improvement and optimization steps for the end user.

3. Standardized Performance for a Manufacturing Scale Bioreactor Train

For this final case study, the requirement was to define a set of process parameters for mAb production in CHO cells across a production bioreactor train of stirred tanks with working volumes of 150, 750 and 3000L. The experiment had to take place within a pre-determined design space for gassing and agitator speed limits, with the requirement for generating a k_La value of at least $15h^{-1}$.

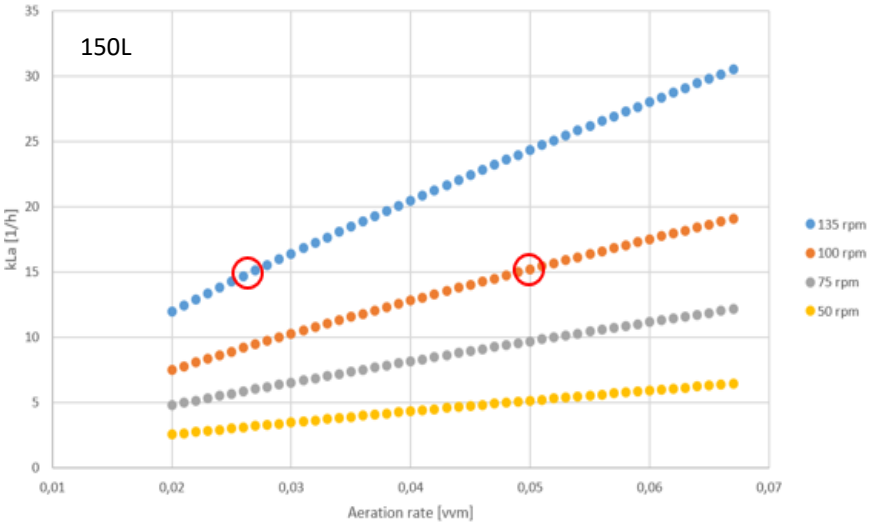
The bioreactors were geometrically similar, all having the same H:D ratio, D:d ratio and sparger type. They also utilized the same agitator design, each having a 2-stage segment type impeller fitted.

It was observed that no inhomogenous mixing or zoning occurred which allowed for accurate modelling. Under standard operating conditions, i.e. within design space parameters, the maximum k_La achievable was more than double the required value at $33h^{-1}$.

For all the 3 bioreactor sizes a hydrodynamical model (Fig. 12) was developed to predict the performance parameter k_La at different process parameters (Fig.13).

$$k_L a = K \left(\frac{P_g}{V}\right)^{\alpha} \dot{v}_G{}^{\beta}$$

Fig.12. Model generation for k_La prediction: the exponents α, β and the multiplicator *K* need to be determined with empirical data, obtained from k_La experiments.



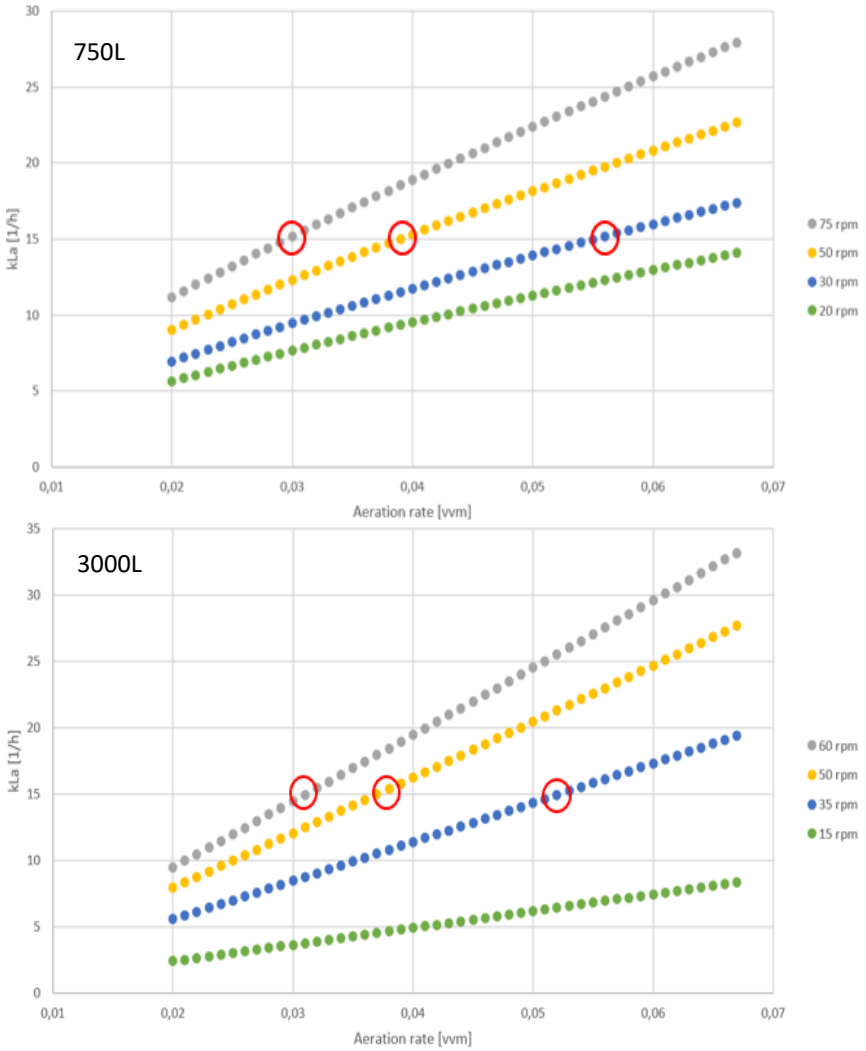


Fig.13. k_La values predicted against increasing aeration rates for varying agitator speeds in 150, 750 and 3000L bioreactors.

Utilizing this model, at 150L, it can be seen that the required k_La value could be reached using two of the power inputs modelled: 165W/m³ and 400W/m³. Within the functional limits of the system a $15h^{-1}$ k_La value could be achieved at 400W/m³ at approximately half the aeration rate of that required at 165W/m³ (0.05vvm).

At the 750L scale, the desired k_La value could be reached with three of the four power inputs tested: 13, 45 and 204W/m³. Similarly, this could also be achieved at the 3000L with 50, 145 and 250W/m³.

As the required k_La value was achievable within the configurable limits of the system, the end user was able create an improved output from all three vessels and maintain a fixed superficial gas velocity of at least $7*10^{-3}$ m/sec, across the whole bioreactor train, to standardize the production process, reduce the variation in parameters and make it easier to control.

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