# Engineering characterization of the single-use Xcellerex XDR-200 stirred-tank bioreactor system

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# Engineering characterization of the single-use Xcellerex™ XDR-200 stirred-tank bioreactor system

**This application note describes the physical characteristics of the XDR-200 bioreactor system suitable for use in mammalian cell culture applications. The presented data can be useful in process transfer, scale-up, and comparison of different bioreactor systems.**

## Introduction

For both conventional and single-use bioreactor systems, the knowledge of physical parameters such as $k_La$ and mixing time is important for scaling and transferring of processes between different types of bioreactors. Characterization data is also useful in determination of a system's suitability for intended applications.

The single-use XDR-200 stirred-tank bioreactor system can be used for various cell culture applications. The aim of this study was to give a detailed description of the physical characteristics of XDR-200 in terms of mixing time, heating and cooling time, and $k_La$ when equipped with a single-use XDA 200 L cell culture bag assembly.

## Materials and methods

### System setup

An XDR-200 system was equipped with an air-cooled temperature control unit (TCU) with 9 kW heating and 1.5 HP cooling capacity. For temperature measurements, a standard XDR resistance temperature detector (RTD) was used. A single-use XDA 200 L cell culture development bag assembly, with integral pitched-blade impeller as well as macro- (0.5 and 1 mm) and micro- (2 and 20 µm) spargers, was used for determination of mixing time, heating and cooling time, and $k_La$.

### Mixing Time

Mixing time was evaluated by determining the time required to reach 95% of a pH step change ($t_{m95}$) (Fig 1). The XDA 200 L cell culture development bag was filled with phosphate buffered saline (PBS). The pH shift was generated by adding a shot of acid (0.5 M HCl in PBS) from the top of the bioreactor into the liquid at a ratio of 1:1000 to the liquid volume. The pH was recorded using external pH probes positioned in the bioreactor as depicted in Figure 2. To establish starting conditions, base (0.5 M NaOH in PBS) was added at a ratio of 1:1000 to the liquid volume after each mixing time experiment. Bioreactor content, corresponding to the volume of added acid and base, was regularly removed from the bulk liquid to ensure constant XDR test volume. Mixing time experiments were run at three different volume and agitation rate settings: 40, 120, 200 L and 30, 190, and 350 rpm, respectively. The temperature was controlled at 37°C and the impeller was run counterclockwise to provide an upward fluid flow.

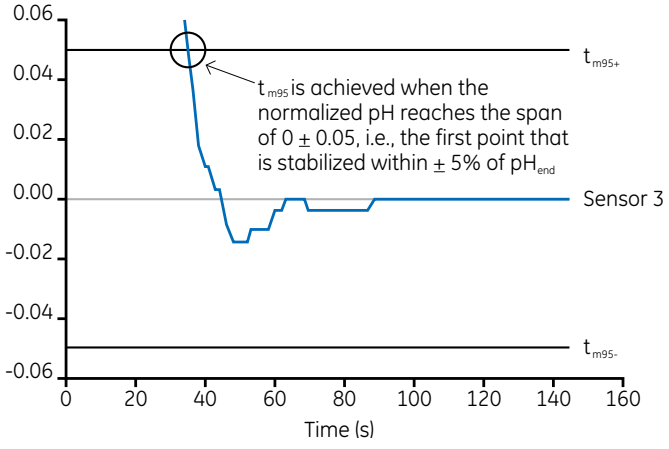


**Fig 1.** Example of normalized pH data for $t_{m95}$ determination. The black lines indicate pH interval where $t_{m95}$ is achieved.

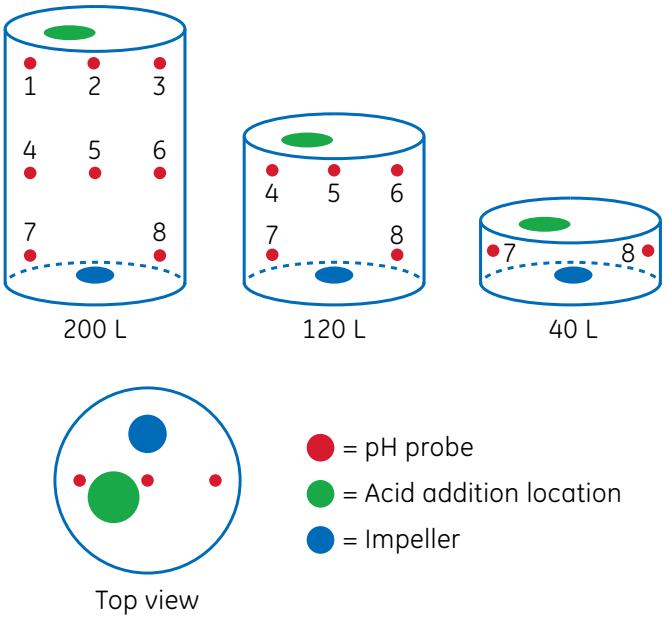


**Fig 2.** The pH probe positions for the tested volumes. Eight probes in three levels were used to record the pH at max. working volume, 200 L. At 120 L, the top probe layer was removed and the pH was recorded by using five probes in two levels. At 40 L, the pH probes of the midsection were removed, leaving the bottom pH probes. Acid and base were added at the top of the bioreactor.

### *Heating-cooling*

The heating-cooling response was assessed by calculating the time to reach 95% of the temperature step change ($t_{95}$), for the temperature intervals 5°C to 20°C, 20°C to 37°C, and 37°C to 5°C, testing three different working volumes: 40, 120, and 200 L. The XDA 200 L cell culture bag was filled with a saline solution consisting of 6 g/L of NaCl dissolved in purified water. The upward fluid flow was held constant at an agitation rate of 175 rpm, as agitation will not affect heating time significantly above a certain threshold. The vessel temperature control PID parameters were set in accordance with factory default settings (P = 4, I = 30, D = 0, DB = 0).

### *Volumetric oxygen transfer coefficient ($k_La$)*

The experiments were set up in accordance with design of experiments (DoE), using a central composite design (CCD) where volume, agitation, and air flow rate were altered to varying levels. Before experiments were initiated, the dissolved oxygen (DO) sensors were calibrated and the response time, that is, the time to reach 63% of air saturation, ($t_{63}$) was measured. For the actual $k_La$ measurements, the XDA bags were filled with purified water supplemented with 6 g/L NaCl, 1 g/L poloxamer 188 (BioChemica), and 50 ppm active simethicone (Antifoam C, Sigma). Testing was performed at three liquid volumes of 40, 120, and 200 L, and the temperature was controlled at 37°C to simulate typical culture conditions. Agitation was varied at 30, 110, and 190 rpm in the up-flow direction. Air flow rate was varied in three levels: 0.5, 2.75, and 5 L/min. To measure DO, two standard XDR DO sensors (Hamilton) were used. All sparger pore sizes (2 µm, 20 µm, 0.5 mm, and 1 mm) were tested individually, using one disc for air transfer in each experiment. The oxygen was depleted from the liquid by addition of nitrogen gas and the DO response was recorded from the time when air flow and agitation had been started. From the recorded DO data, the $k_La$ coefficient could be determined by plotting $ln(DO^* - DO_t)$ as a function of time ($t - t_0$), for which the negative slope yielded the $k_La$ coefficient according to the following equation:

***[Eq. 1]:*** $$ln(DO^* - DO_t) = -k_La(t - t_0) + ln(DO^* - DO_{t0})$$

Where:

$DO^*$ = DO value in equilibrium with the gas bubble concentration, that is, the stabilized value after the measurement is finished.

$DO_t$ = DO value at time t.
$t_0$ = time when the measurement is started.
$DO_{t0}$ = DO value when the measurement is started.

After finalizing the experiments, the obtained $k_La$ coefficients was modelled in the DoE software, MODDE™ version 11.0.0.1717 (Umetrics AB), giving the possibility to assess $k_La$ values for any volume, agitation, and air flow setting within the tested ranges.

## Results

### *Mixing Time*

In Figure 3, results from the mixing time experiments are shown. Only data from the probe position generating the longest $t_{m95}$ for each experiment is plotted to display the worst-case scenario. The shortest $t_{m95}$ was determined to 10 s when measured at 350 rpm and 120 L, whereas the longest $t_{m95}$ was determined to 186 s when measured at 30 rpm and 200 L. The unexpected and relatively long $t_{m95}$ observed at 40 L and 350 rpm was likely caused by vortex formation that disturbed the pH reading in these experiments. Thus, it is likely that the actual shortest $t_{m95}$ is in fact achieved at the lowest volume and highest speed setting.

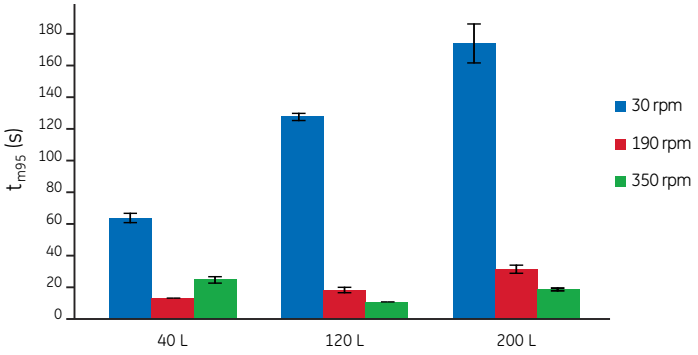


**Fig 3.** Average mixing time ($t_{m95}$) from the probe positions that generated the longest mixing time for each experiment. Error bars correspond to one standard deviation.

Figure 4 displays the $t_{m95}$ (average of duplicate experiments) for each probe position for experiments performed at maximum working volume (200 L) to show how the $t_{m95}$ varies depending on position in the bioreactor. Taking all experiments into account, the average difference in $t_{m95}$ between the probe position resulting in the longest and shortest mixing time was 17 s, indicating effective mixing across the whole bioreactor.

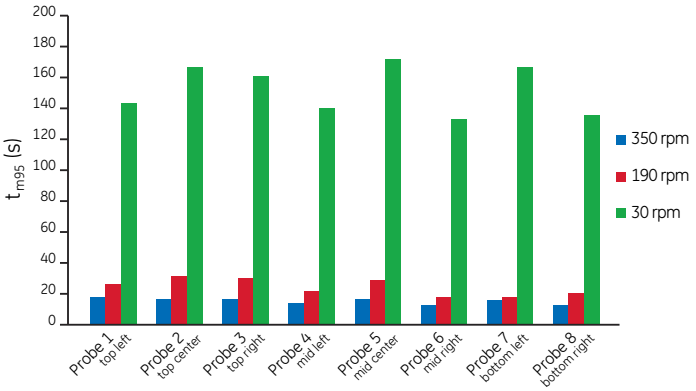


**Fig 4.** Mixing time ($t_{m95}$) for each probe position at maximum working volume (200 L), tested at low, mid-point, and high agitation. Results shown are mean values from duplicate runs.

## Heating and cooling

The results from the heating and cooling experiments are shown in Figure 5. The shortest time for temperature change was achieved for heating from 5°C to 20°C, with heating times of less than 1 h for all volumes tested. Cooling the liquid from 37°C to 5°C required the longest time, between 2.4 and 3.0 h depending on the volume. Heating and cooling times were similar across the tested liquid volumes, except for the 37°C to 5°C interval, where the maximum volume resulted in a somewhat longer (~ 30 min) cooling time.

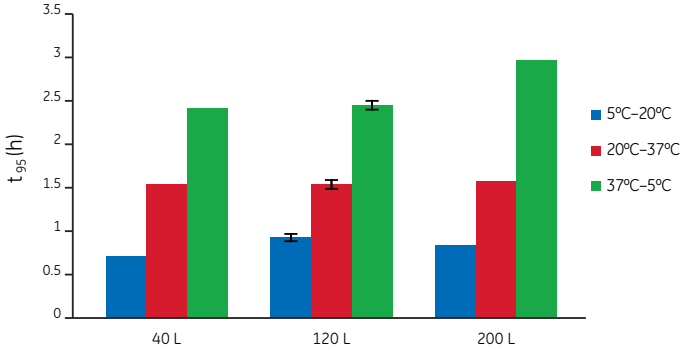


**Fig 5.** Results from the heating-cooling experiments. Error bars for the mid-point volume experiments run in triplicate correspond to one standard deviation.

## Volumetric oxygen transfer coefficient ($k_La$)

Results from $k_La$ determination are shown in Figures 6 to 9 (non-modeled $k_La$ data plotted in bar graphs) and in Figures 10 to 13 (contour plots generated by the DoE software). Measured $k_La$ values ranged from 0.2 to 66 $h^{-1}$. The $k_La$ coefficient was found to increase with agitation and air flow rate. Increasing the liquid volume and sparger pore size had a negative impact on $k_La$. The average response time ($t_{63}$) of the DO probes used was 36 s, which should be sufficient to determine $k_La$ values of acceptable accuracy up to 100 $h^{-1}$ in accordance with $t_{63}$, crit= 1/ $k_La_{max}$ (1).

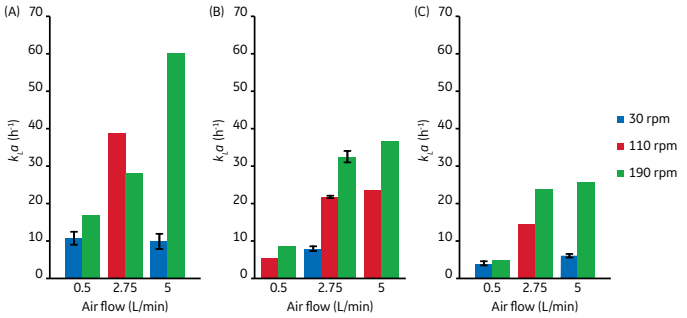


**Fig 6.** Results from the $k_La$ tests for the 2 µm sparger in (A) 40 L, (B), 120 L, and (C) 200 L. The DoE center point for mid-point agitation, volume, and air flow was replicated in triplicate runs (other data points were measured in duplicate). Error bars correspond to one standard deviation.

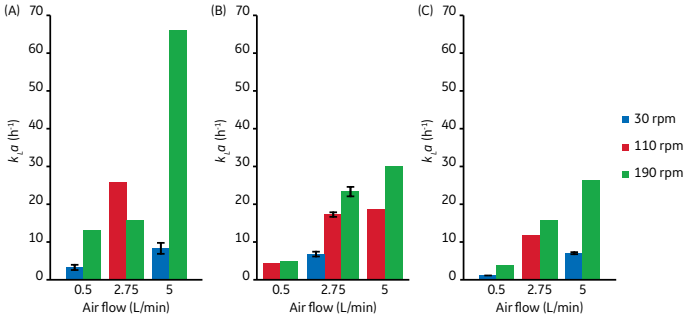


**Fig 7.** Results from the $k_La$ tests for the 20 µm sparger in (A) 40 L, (B), 120 L, and (C) 200 L. The DoE center point for mid-point agitation, volume, and air flow was replicated in triplicate runs (other data points were measured in duplicate). Error bars correspond to one standard deviation.

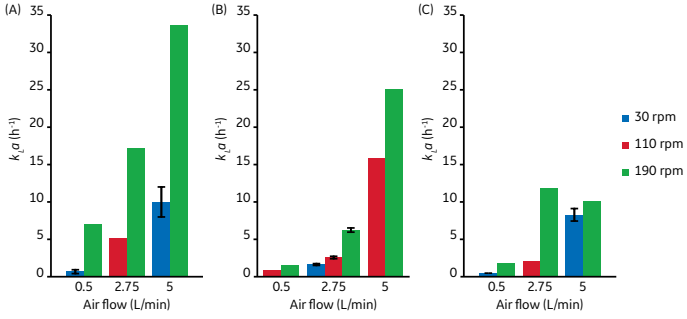


**Fig 8.** Results from the $k_La$ tests for the 0.5 mm sparger in (A) 40 L, (B), 120 L, and (C) 200 L. The DoE center point for mid-point agitation, volume, and air flow was replicated in triplicate runs (other data points were measured in duplicate). Error bars correspond to one standard deviation.

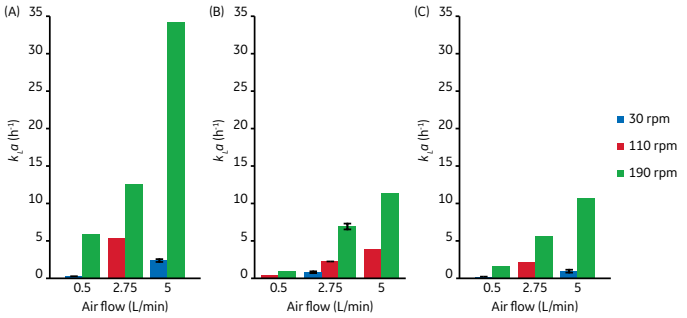


**Fig 9.** Results from the $k_La$ tests for the 1 mm sparger in (A) 40 L, (B), 120 L, and (C) 200 L. The DoE center point for mid-point agitation, volume, and air flow was replicated in triplicate runs (other data points were measured in duplicate). Error bars correspond to one standard deviation.

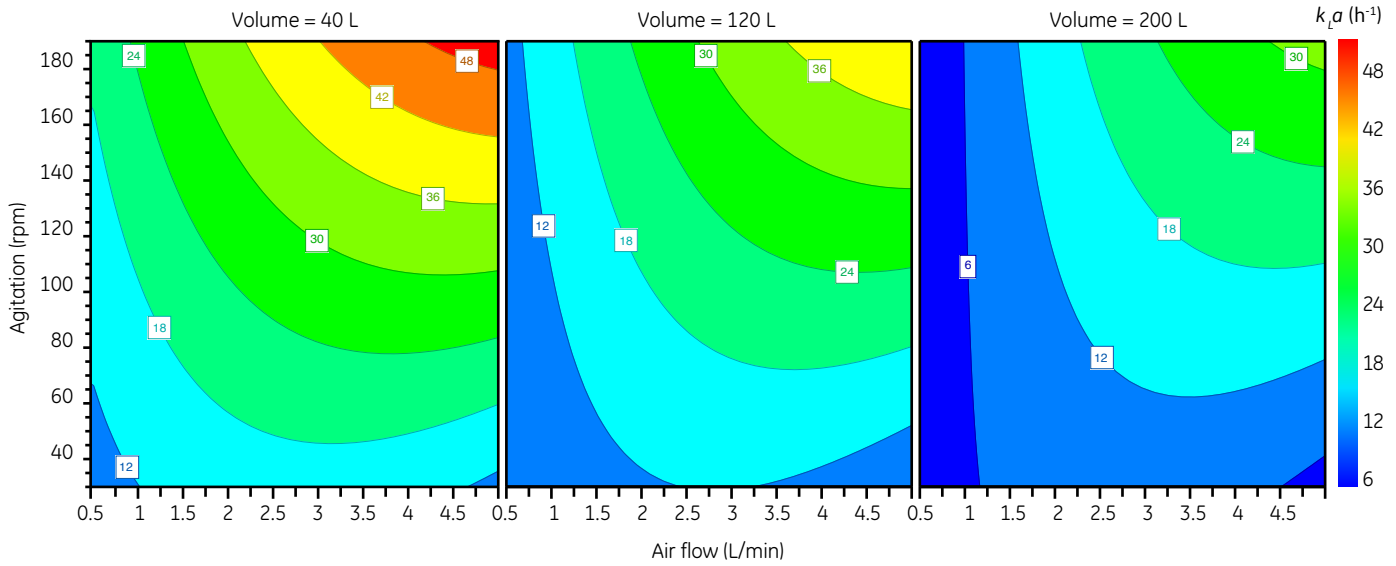


**Fig 10.** 4D response contour plot displaying $k_La$ obtained with the 2 µm sparger pore size at varying air flow, volume, and agitation. The model fit ($R^2$) is 0.90, the model predictability ($Q^2$) is 0.79, and the residual standard deviation (RSD) of the model is 4.97 $h^{-1}$.

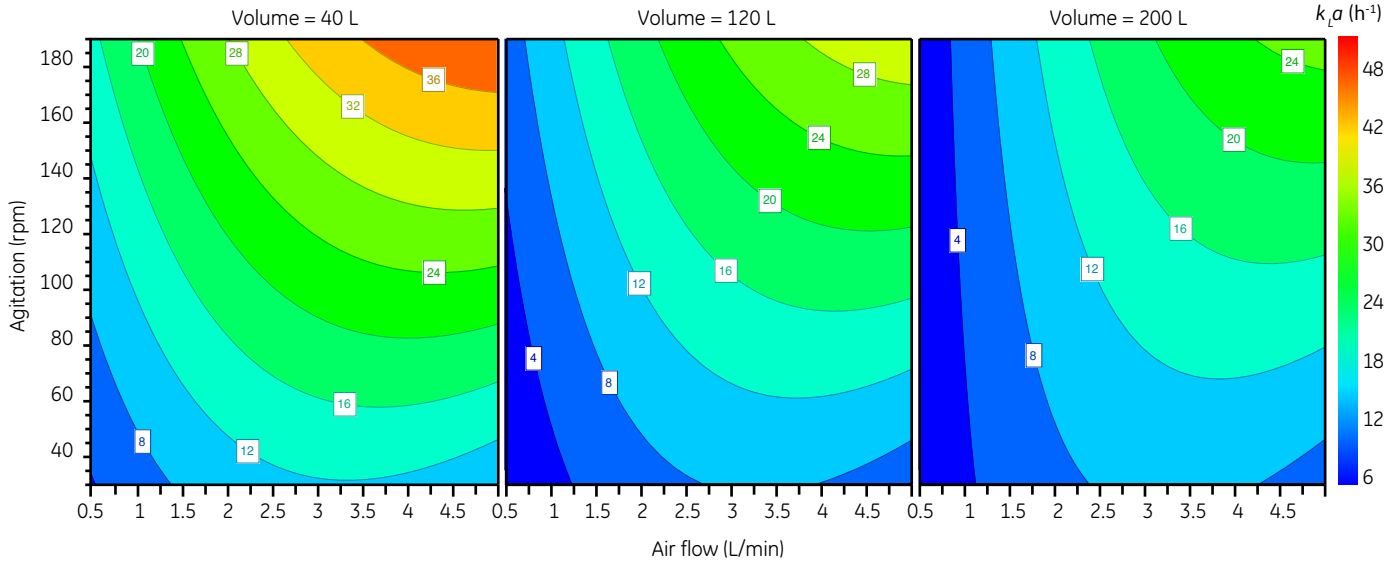


**Fig 11.** 4D response contour plot displaying $k_La$ obtained with the 20 µm sparger pore size at varying air flow, volume, and agitation. The model fit ($R^2$) is 0.97, the model predictability ($Q^2$) is 0.91, and the residual standard deviation (RSD) of the model is 1.86 $h^{-1}$.

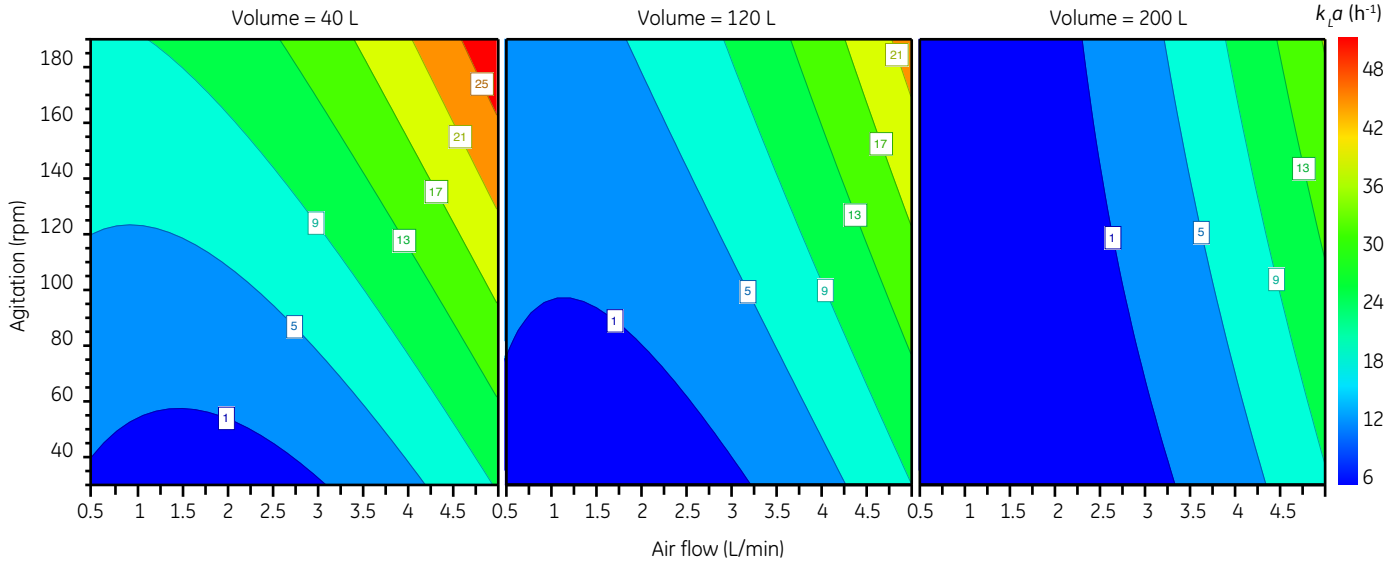


**Fig 12.** 4D response contour plot displaying $k_La$ obtained with the 0.5 mm sparger pore size at varying air flow, volume, and agitation. The model fit ($R^2$) is 0.85, the model predictability ($Q^2$) is 0.67, and the residual standard deviation (RSD) of the model is 3.39 $h^{-1}$.

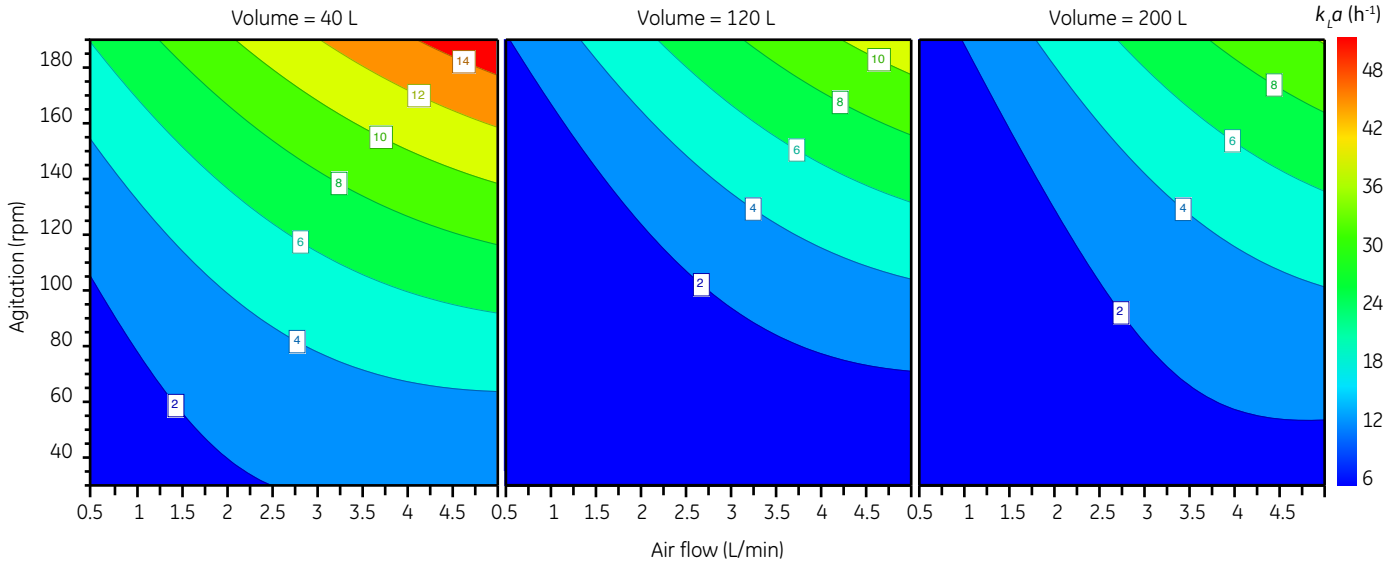


**Fig 13.** 4D response contour plot displaying $k_La$ obtained with the 1.0 mm sparger pore size at varying air flow, volume, and agitation. The model fit ($R^2$) is 0.98, the model predictability ($Q^2$) is 0.94, and the residual standard deviation (RSD) of the model is 0.66 $h^{-1}$.

## Conclusions

This application note gives a detailed description of the physical characteristics of the XDR-200 stirred-tank bioreactor system in terms of mixing time, heating and cooling time, and $k_La$. Mixing time results show sufficient mixing capacity to ensure a well-mixed tank. Measures such as heating and cooling capability and volumetric oxygen transfer coefficient were determined within ranges typical for culture applications, and obtained values were found to meet the requirements of commonly used cells. The generated information can be used for process transfer, scale-up, and comparison of different bioreactor types.

## Disclaimer

The results from the characterization experiments and the conclusions presented in this application note are valid for this specific study. Other study conditions could have significant impact on the outcome. For each parameter, certain variability in the result can be expected depending on choice of method, measuring equipment, and test settings such as temperature and liquid composition.

## Reference

1. Van't Riet, K. Review of measuring methods and results in nonviscous gas-liquid mass transfer in stirred vessels. *Ind Eng Chem Process Des Dev* **18**, 357–364 (1979).

## Ordering information

| Product | Description | Product code |
|---|---|---|
| XDA-200 development bioreactor bag | 200 L, pitch blade impeller, one disc each of 2 µm, 20 µm, 1 mm spargers and two discs with 0.5 mm orifice diameter. | 888-0151-C |

To order the XDR-200 system, please contact your local sales representative.

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