



# Engineering characterization of the single-use Xcellerex XDR-1000 stirred-tank bioreactor system

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# Engineering characterization of the single-use Xcellerex™ XDR-1000 stirred-tank bioreactor system

**This application note describes the physical characteristics of the XDR-1000 bioreactor system suitable for use in mammalian cell culture applications. The presented data can be useful in process transfer and comparison of different bioreactor systems.**

## Introduction

For both conventional and single-use bioreactor systems, the knowledge of physical parameters such as  $k_La$ , mixing time, and power input is important for 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-1000 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-1000 in terms of mixing time, heating and cooling times, power input, and  $k_La$  when equipped with a single-use XDA 1000 L cell culture bag assembly.

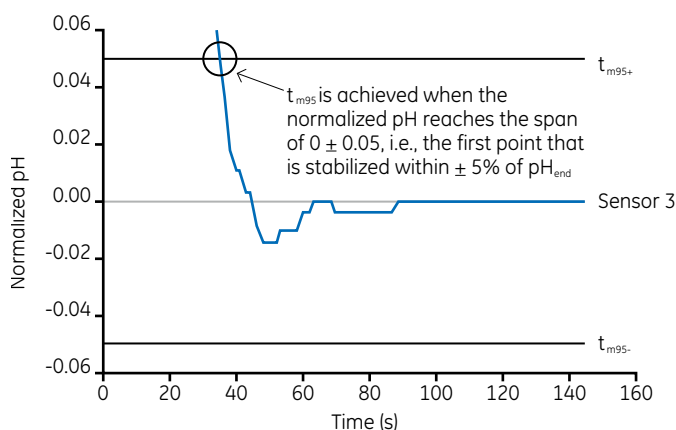
## Materials and methods

### System setup

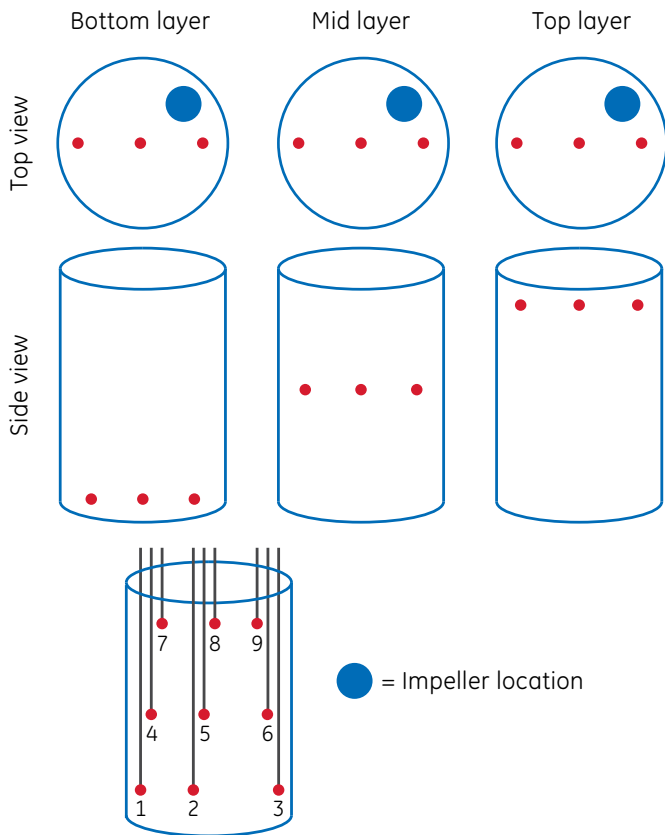
The XDR-1000 system was equipped with an air-cooled temperature control unit (TCU) with 9 kW heating and 1.5 HP cooling capacity. A single-use XDA 1000 L cell culture Pro bag assembly with an integral pitched-blade impeller and eight discs of micro spargers with 2  $\mu\text{m}$  pore size was used for determination of mixing time, heating and cooling times, power input, and  $k_La$ . The exhaust filter of the bioreactor was equipped with the included filter heater. For temperature measurements, a standard XDR resistance temperature detector (RTD) was used.

### 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 1000 L cell culture Pro bag was filled with phosphate buffered saline (PBS). The pH shift was generated by adding acid (0.5 M HCl in PBS) in one shot from the top of the bioreactor into the liquid at a ratio of 1:1000 to the liquid volume. The pH was recorded using nine 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 removed from the bulk liquid after each test run to ensure constant test volume. Mixing time experiments were run at three different volume and agitation rate settings: 200, 600, 1000 L and 15, 77.5, and 140 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 the interval where  $t_{m95}$  is achieved.



**Fig 2.** The pH probe positions at the maximum working volume (1000 L). At 600 L, the mid and top probe layers were lowered proportionally with the fluid to capture the three levels within the smaller volume. At 200 L, the pH probes of the midsection were removed and the pH was recorded by using six probes in two levels. Acid and base were added from the top as close to the center as possible for all experiments.

## Heating and 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: 200, 600, and 1000 L. The XDA 1000 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 100 rpm, as agitation would not affect heating time significantly above a certain threshold. The vessel temperature control PID parameters were set in accordance with factory default settings ( $P = 4$ ,  $I = 30$ ,  $D = 0$ ,  $DB = 0$ ).

## Power input

Power input to the XDR-1000 system was assessed by measuring the motor current at agitation rates between 20 and 140 rpm (upward fluid flow) under gassed (10 L/min) and ungassed conditions. The XDA 1000 L cell culture bag was filled with 6 g/L NaCl, 1 g/L poloxamer 188, and 50 ppm active silicone (Antifoam C, Sigma) in purified water to a volume of 1000 L. Temperature was controlled at 37°C. The motor current was recorded from the servo drive of the

XDR-1000 system and converted into torque using the torque constant of 2.04 ( $\pm 10\%$ ) Nm/A (Kollmorgen, Radford, VA, USA). To compensate for the power loss due to friction in the motor and impeller assembly, power was measured during zero load conditions for each tested agitation setting. The zero load condition was established by wetting the impeller bearing without submerging the impeller blades.

Power input ( $P$ ) was calculated from the motor current readings using the following equation:

$$\text{[Eq. 1]: } P = (I_{\text{load}} - I_{\text{zero load}}) * K_T * 2\pi N$$

To determine the power number ( $N_p$ ), the following equation was used:

$$\text{[Eq. 2]: } N_p = \frac{P_0}{N^3 * D_i^5 * \rho}$$

Reynolds number ( $N_{Re}$ ) was calculated using the following equation:

$$\text{[Eq. 3]: } N_p = \frac{N * D_i^2 * \rho}{\mu}$$

Where:

$P$  = power input in W

$I_{\text{load}}$  = motor current at load conditions in A

$I_{\text{zero load}}$  = motor current at zero load conditions in A

$K_T$  = bioreactor's torque constant in Nm/A

$P_0$  = power input in non-aerated liquid in W

$N$  = impeller speed in  $s^{-1}$

$D_i$  = impeller diameter in m (0.323 m for XDR-1000)

$\rho$  = medium density in  $kg/m^3$  (here 1000  $kg/m^3$  was used)

$\mu$  = medium viscosity in  $kg/m/s$  (here 0.001  $kg/m/s$  was used)

## Volumetric oxygen transfer coefficient ( $k_L a$ )

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) sensor was calibrated. For the actual  $k_L a$  measurements, the XDA bags were filled with purified water supplemented with 6 g/L NaCl, 1 g/L poloxamer 188, and 50 ppm active silicone (Antifoam C, Sigma). Testing was performed at three liquid volumes of 200, 600, and 1000 L, and the temperature was controlled at 37°C to simulate typical culture conditions. Agitation was varied between 15, 77.5, and 140 rpm in the up-flow direction. Air flow rate was varied between three levels: 1, 5.5, and 10 L/min. To measure DO, a standard XDR DO sensor (Hamilton) was used. All eight sparger discs with 2  $\mu m$  pore size were tested simultaneously 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  $k_L a$  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  $k_L a$  coefficient according to the following equation:

$$\text{[Eq. 4]: } \ln(DO^* - DO_t) = -k_L a(t - t_0) + \ln(DO^* - DO_{t_0})$$

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.

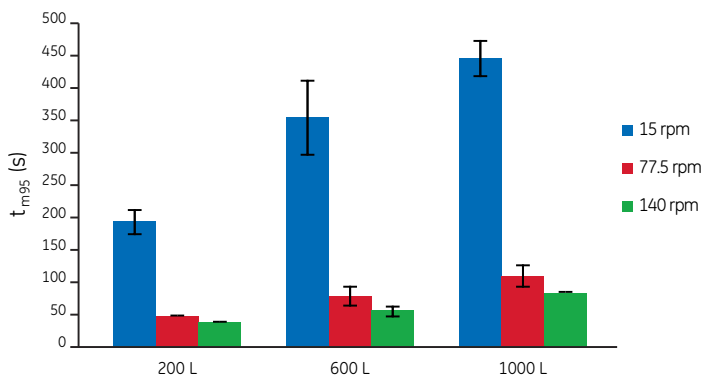
$DO_{t_0}$  = DO value when the measurement is started.

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

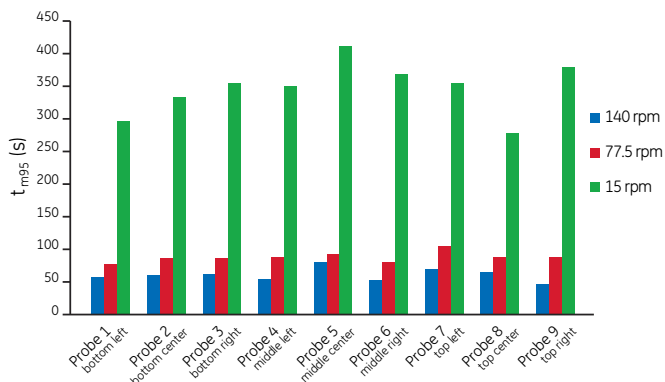
## Results

### 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 run is plotted to display the worst-case scenario. The shortest  $t_{m95}$  was determined to 36.5 s when measured at 200 L and 140 rpm, whereas the longest  $t_{m95}$  was determined to 473.5 s when measured at 1000 L and 15 rpm. Figure 4 displays the  $t_{m95}$  (average of duplicate experiments) for each probe position for experiments performed at maximum working volume (1000 L) to show how the mixing time 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 68 s, indicating effective mixing across the whole bioreactor.



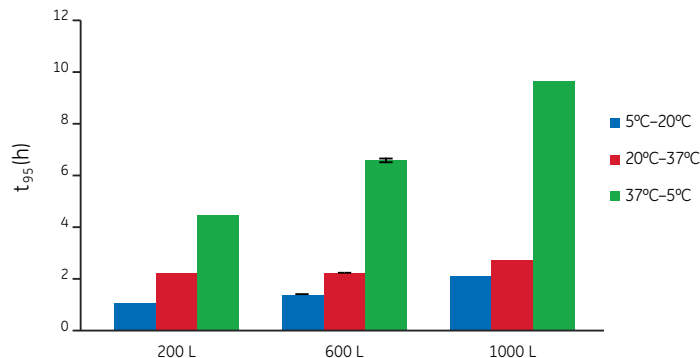
**Fig 3.** Average mixing times ( $t_{m95}$ ) from the probe positions that generated the longest mixing time for all tested volumes and agitation rates. Error bars correspond to one standard deviation.



**Fig 4.** Mixing time ( $t_{m95}$ ) for each probe position for maximum working volume (1000 L), tested at low, mid-point, and 140 rpm. Results are averaged 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 2.1 h for all volumes tested. Cooling the liquid from 37°C to 5°C required the longest time, between 4.5 and 9.7 h depending on the volume. In general, the time required for heating and cooling was found to increase with liquid volume.

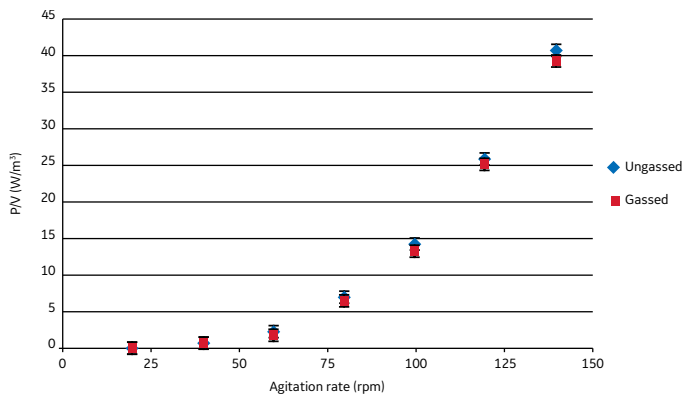


**Fig 5.** Results from the heating-cooling experiments. Error bars correspond to one standard deviation.

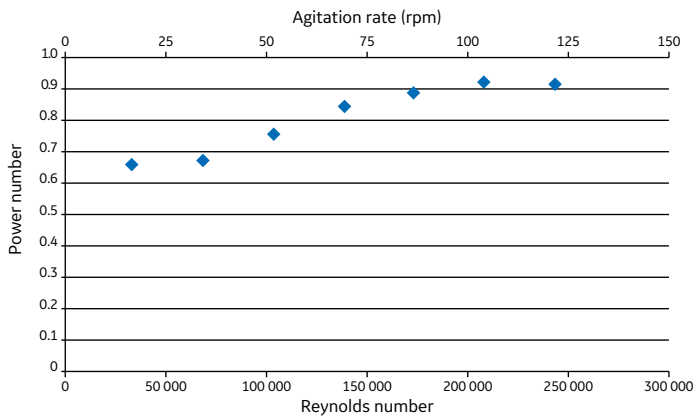
### Power input

Figure 6 shows volumetric power input at maximum working volume (1000 L) plotted against agitation rate for gassed (10 L/min) and ungassed conditions. The power input was found marginally lower for gassed conditions compared with ungassed conditions, with an average  $P_g/P_0$  corresponding to 0.87 (power input obtained at 20 rpm excluded).

In Figure 7, calculated power number from experimental power input data is plotted against Reynolds number and agitation rate. At agitation rates above 80 rpm, the calculated power number remains nearly constant with an average value of approximately 0.9.



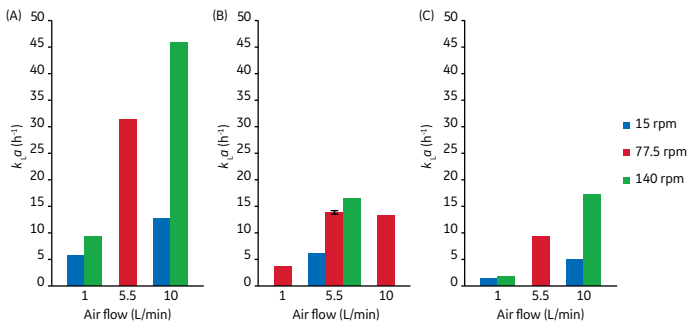
**Fig 6.** Power input per liquid volume plotted against agitation rate at 1000 L working volume. The air flow was 10 L/min for gassed conditions. Error bars correspond to one standard deviation.



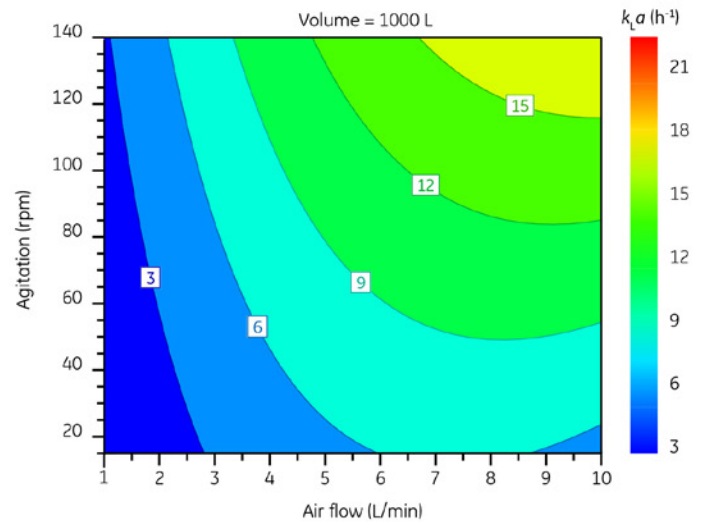
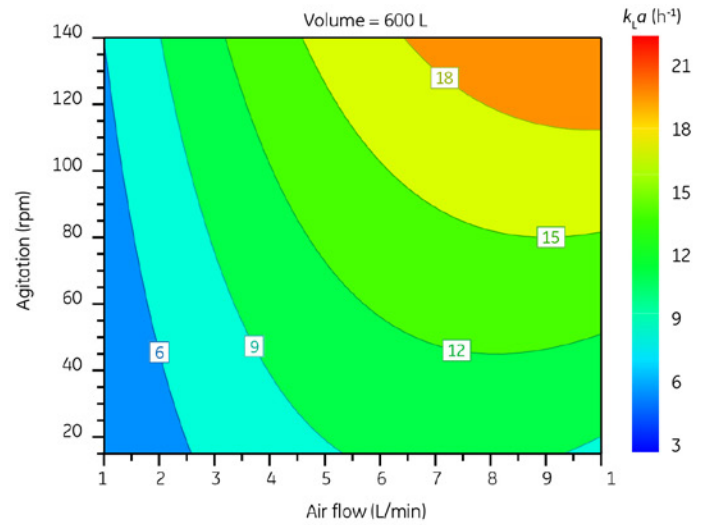
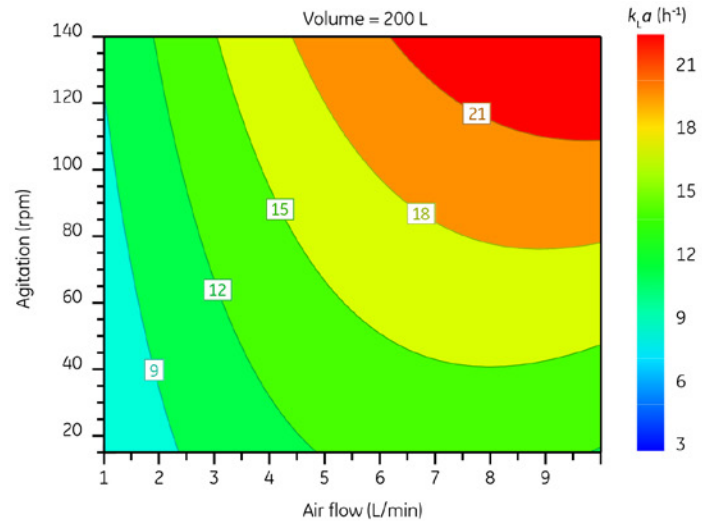
**Fig 7.** Power and Reynolds numbers calculated from the measurements shown in Figure 6.

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

Results from  $k_La$  determination are shown in Figure 8 (non-modelled  $k_La$  data plotted in bar graphs) and Figure 9 (contour plot generated by the DoE software from experimental data). Measured  $k_La$  values ranged from 1.5 to 46  $h^{-1}$ . The  $k_La$  value was found to increase with agitation and air flow rate. Increasing the liquid volume had a negative impact on  $k_La$ . Data from two experiments performed at high gas flow and agitation in 200 L liquid volume, which are conditions out of range for most cell cultures, were excluded from the DoE model as they would have skewed the overall fit. Hence, the contour plots displayed for 200 L in Figure 9 might underestimate the  $k_La$  value to some extent.



**Fig 8.** Results from the  $k_La$  tests for the 2  $\mu m$  sparger in (A) 200 L, (B) 600 L, and (C) 1000 L. The DoE center point was replicated in triplicate runs. The error bar corresponds to one standard deviation.



**Fig 9.** 4D response contour plot displaying  $k_La$  obtained with the 2  $\mu m$  sparger pore size at varying air flow, volume, and agitation. The model fit ( $R^2$ ) is 0.95, the model predictability ( $Q^2$ ) is 0.87, and the residual standard deviation (RSD) of the model is 1.48  $h^{-1}$ .

## Conclusions

This application note gives a detailed description of the physical characteristics of the XDR-1000 stirred-tank bioreactor system in terms of mixing time, heating and cooling times, power input, and  $k_La$ . Mixing time results show sufficient mixing capacity to ensure a well-mixed tank. Heating and cooling capability, power input, and volumetric oxygen transfer coefficients 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 results can be expected depending on choice of method, measuring equipment, and test conditions such as temperature and liquid composition.

## Ordering information

Product	Description	Product code
XDA-1000 Pro bioreactor bag	1000 L, pitch blade impeller, and eight discs of micro spargers (2 $\mu$ m)	888-0071-C

To order the XDR-1000 system, please contact your local sales representative.







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