Engineering characterization of the single-use Xcellerex XDR-50 stirred-tank bioreactor system

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Engineering characterization of the single-use Xcellerex™ XDR-50 stirred-tank bioreactor system

This application note describes the physical characteristics of the XDR-50 bioreactor system suitable for use in both mammalian cell culture and microbial fermentation 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 and mixing time 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 dual-purpose single-use XDR-50 stirred-tank bioreactor system can be used interchangeably for either cell culture or microbial fermentation simply by adding or removing predefined accessories and by using an application-specific XDA disposable bag assembly. Accessories for microbial fermentation include larger exhaust gas filter heater, exhaust gas condenser, and a set of three baffles. These accessories, along with the XDA fermentor bag and an exhaust condenser bag, constitute a highly adaptable fermentation platform for bioprocessing applications.

The aim of this study was to give a detailed description of the physical characteristics of XDR-50 in terms of mixing time, heating and cooling time, and k_La when equipped with a single-use XDA 50 L cell culture bag assembly. In addition, maximum oxygen transfer rate (OTR_{max}) for the XDR-50 system, equipped with a single-use XDA fermentor bag assembly, was determined.

Materials and methods

System setup

An XDR-50 system was equipped with an air-cooled temperature control unit (TCU) with 3 kW heating and 1 HP cooling capacity. For temperature measurements, a standard XDR resistance temperature detector (RTD) was used. A single-use XDA 50 L cell culture development bag assembly, with an 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 times, and k_La.

To test OTR_{max} for microbial fermentation applications, a single-use XDA 50 L fermentor bag, with an integral two-stage impeller assembly, comprising a six-blade Rushton impeller and a six-blade/40° axial flow impeller, was used. The sparger consisted of eight open tube spargers and the fermentor was further equipped with three 32 × 1.2 inch baffles, an exhaust gas condenser, and matching exhaust filter heaters.

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 50 L cell culture development 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 six pH probes positioned in the bioreactor as depicted in Figure 2. In addition to the standard XDR pH probe (Hamilton), five external pH probes were used. 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: 15, 32.5, 50 L and 40, 200, and 360 rpm, respectively. The temperature was controlled at 37°C and the impeller was run counterclockwise to provide an upward fluid flow.

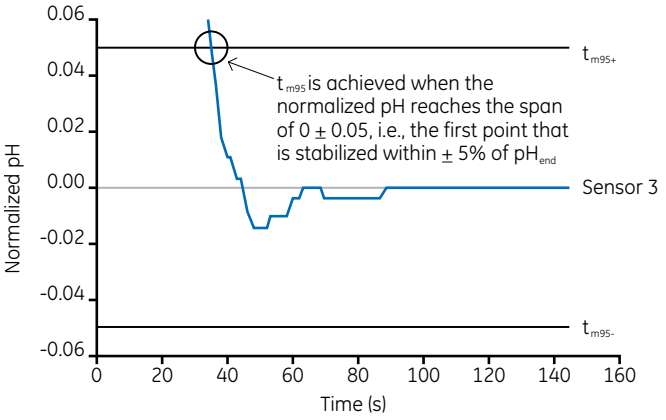

Fig 1. Example of normalized pH data for t_{m95} determination. The black lines indicate the interval where t_{m95} is achieved.

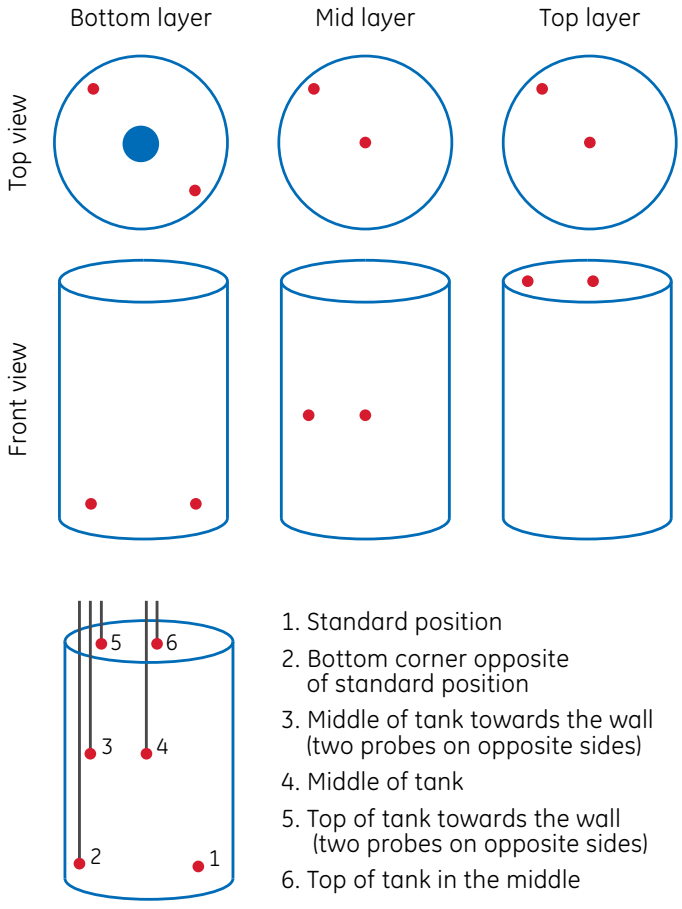

Fig 2. The pH probe positions at maximum working volume (50 L). At 32.5 L, probe number 5 and 6 were lowered as much as the water level and probe number 3 was lowered to be positioned in between probes 5/6 and 2. Probe 4 was disconnected. At 15 L, all probes except number 1 and 2 were disconnected. The acid and base were added at the top as close to the center as possible.

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: 15, 32.5, and 50 L. The XDA 50 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 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).

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 silicone (Antifoam C, Sigma). Testing was performed at three liquid volumes of 15, 32.5 and 50 L, and the temperature was controlled at 37°C to simulate typical culture conditions. Agitation was varied between 40, 120, and 200 rpm in the up-flow direction. Air flow rate was varied between three levels: 0.25, 2.625, 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.

Maximum oxygen transfer rate (OTR_{max})

The sodium sulfite method was used to determine the maximum oxygen transfer rate at three different working volumes, using both agitation directions (up-flow and down-flow), at maximum agitation rate, and with a gas flow rate that is varied with liquid volume to retain the same volume of gas per volume of liquid per minute (VVM). The measuring principle is based on the reaction rate when sulfite is oxidized to sulfate, from which the oxygen transfer rate can be deduced. A fixed amount of sodium sulfite is added to the bioreactor liquid together with a catalyst, typically copper sulfate, while maintaining the desired process conditions in terms of temperature, agitation, and air flow. In these experiments the XDA 50 L fermentor bag was filled with purified water to the tested volumes of 15, 32.5, and 50 L. The temperature was controlled at 37°C. The agitation rate was 360 rpm using both up- and downward fluid flow direction, and the gas flow rate was 1 VVM, consisting of a 50%/50% air and oxygen mixture. To start the reaction, deaerated slurry of 17 g/L Na_2SO_3 was added to the fermentor containing the catalyst, 0.3 g/L $CuSO_4 \times 5H_2O$, in purified water. To determine OTR, the time at which the DO was below 50% was recorded (Fig 3). The oxygen flow was started at the time point of the Na_2SO_3-slurry addition.

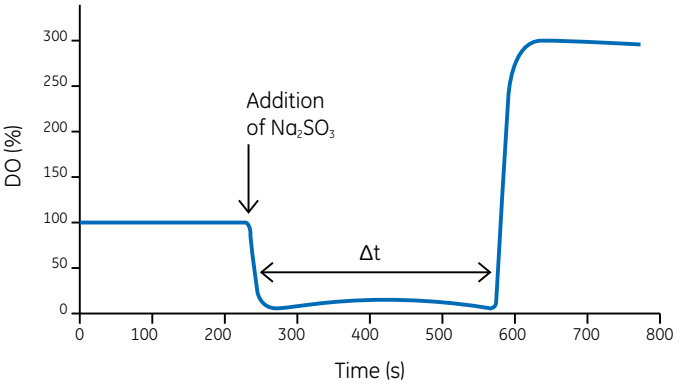

Fig 3. DO curve during OTR measurements. The time when DO is below 50% (Δt) was used for calculating the OTR. The DO reached 300% of air saturation after the reaction was over, as the gas was enriched with 50% oxygen from the time point when Na_2SO_3 was added.

Thereafter, OTR was calculated using the following equation:

[Eq. 2]: $$OTR = \frac{0.5 * n_{Na2SO3}}{V * \Delta t}$$

Where:

n_{NaSO3} = amount of Na_2SO_3 added in moles

V = liquid volume in L

Δt = time when DO is below 50% (Fig 3)

Results

Mixing time

In Figure 4, 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 12 s when measured at 360 rpm and 15 L, whereas the longest t_{m95} was determined to 78 s when measured at 40 rpm and 50 L. Figure 5 displays the t_{m95} (average of duplicate experiments) for each probe position for experiments performed at maximum working volume (50 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 12 s, indicating effective mixing across the whole bioreactor.

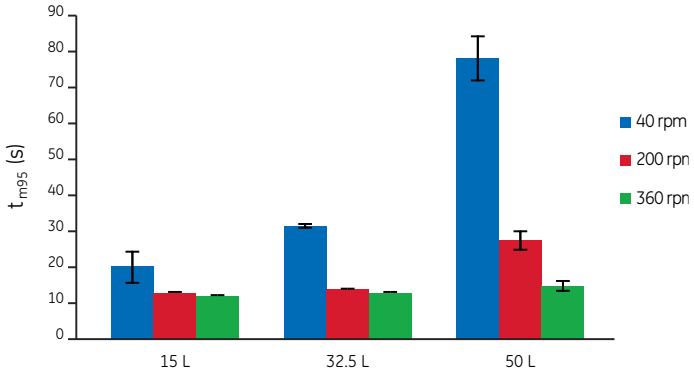

Fig 4. Average mixing times (t_{m95}) from the probe positions that generated the longest mixing time for all tested volumes and agitation rates. Error bars correspond to one standard deviation.

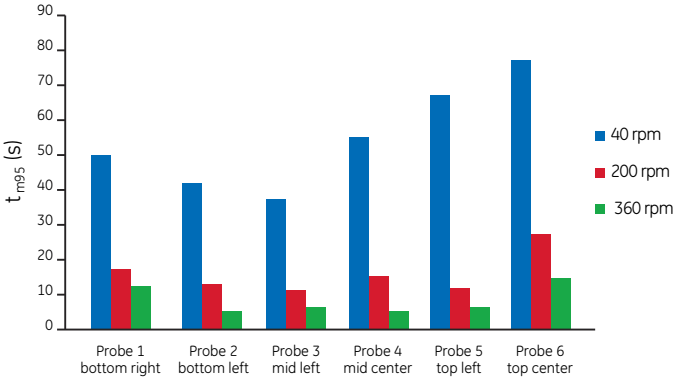

Fig 5. Mixing time (t_{m95}) for each probe position for maximum working volume (50 L), tested at low, mid-point, and high agitation. Results are averaged values from duplicate runs.

Heating and cooling

The results from the heating and cooling experiments are shown in Figure 6. The shortest time for temperature change was achieved for heating from 5°C to 20°C, with heating times of less than 0.8 h for all volumes tested. Cooling the liquid from 37°C to 5°C required the longest time, between 0.8 and 1.8 h depending on the liquid volume. The time required for heating and cooling was similar across the tested liquid volumes, although showing a trend of somewhat longer heating and cooling times for 22 L. This observation can be attributed to the lower heat transfer area-to-volume ratio in the case of 22 L.

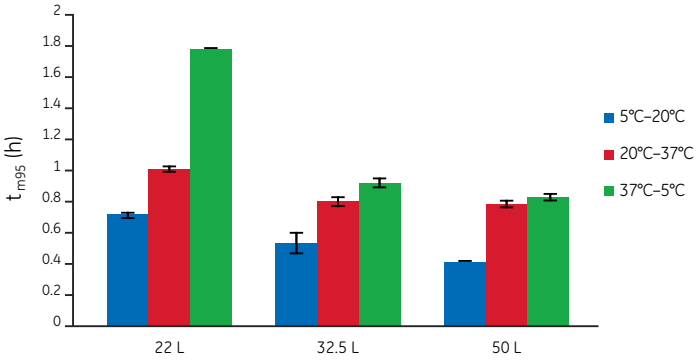

Fig 6. Results from heating-cooling experiments. Error bars correspond to one standard deviation.

Volumetric oxygen transfer coefficient (k_La)

Results from k_La determination are shown in Figures 7 to 10 (non-modelled k_La data plotted in bar graphs) and in Figures 11 to 14 (contour plots generated by the DoE software). Measured k_La values ranged from 0.3 to 105 h^{-1}. The k_La coefficient was found to increase with agitation and air flow rate. Increasing the liquid volume had a slight negative effect. The average response time (t_{63}) of the DO probes used was 16 s, which should be sufficient for determining k_La values of acceptable accuracy up to 225 h^{-1} in accordance with t_{63}, crit= 1/ k_La_{max} (1).

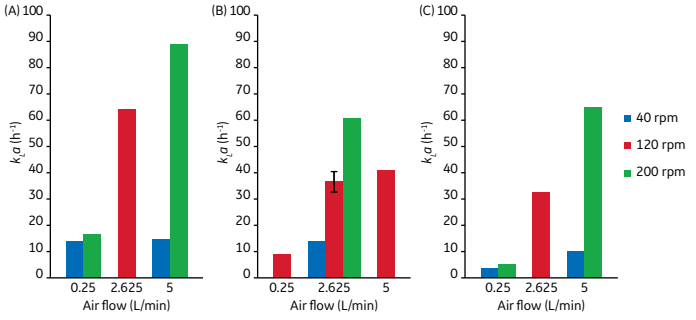

Fig 7. Results from the k_La tests for the 2 µm sparger in (A) 15 L, (B) 32.5 L, and (C) 50 L. The DoE center point was replicated in triplicate runs. The error bar corresponds to one standard deviation.

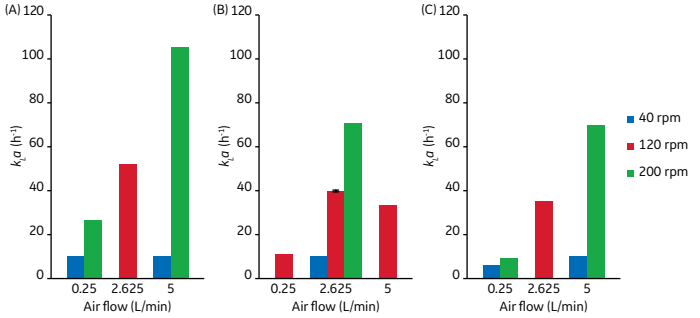

Fig 8. Results from the k_La tests for the 20 µm sparger in (A) 15 L, (B) 32.5 L, and (C) 50 L. The DoE center point was replicated in triplicate runs. The error bar corresponds to one standard deviation.

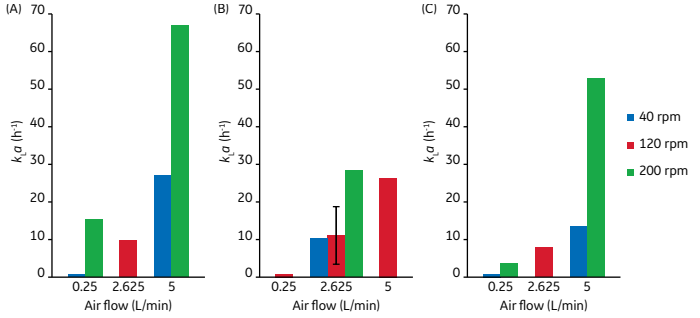

Fig 9. Results from the k_La tests for the 0.5 mm sparger in (A) 15 L, (B) 32.5 L, and (C) 50 L. The DoE center point was replicated in triplicate runs. The error bar corresponds to one standard deviation.

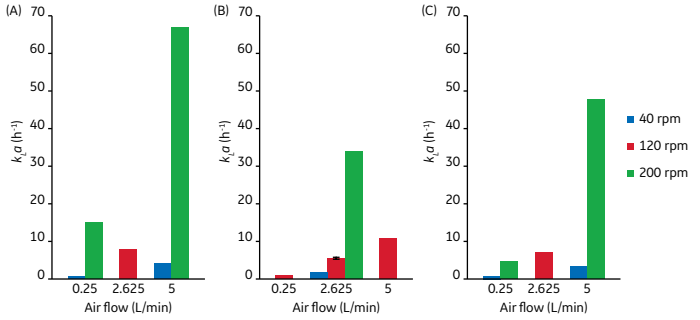

Fig 10. Results from the k_La tests for the 1.0 mm sparger in (A) 15 L, (B) 32.5 L, and (C) 50 L. The DoE center point was replicated in triplicate runs. The error bar corresponds to one standard deviation.

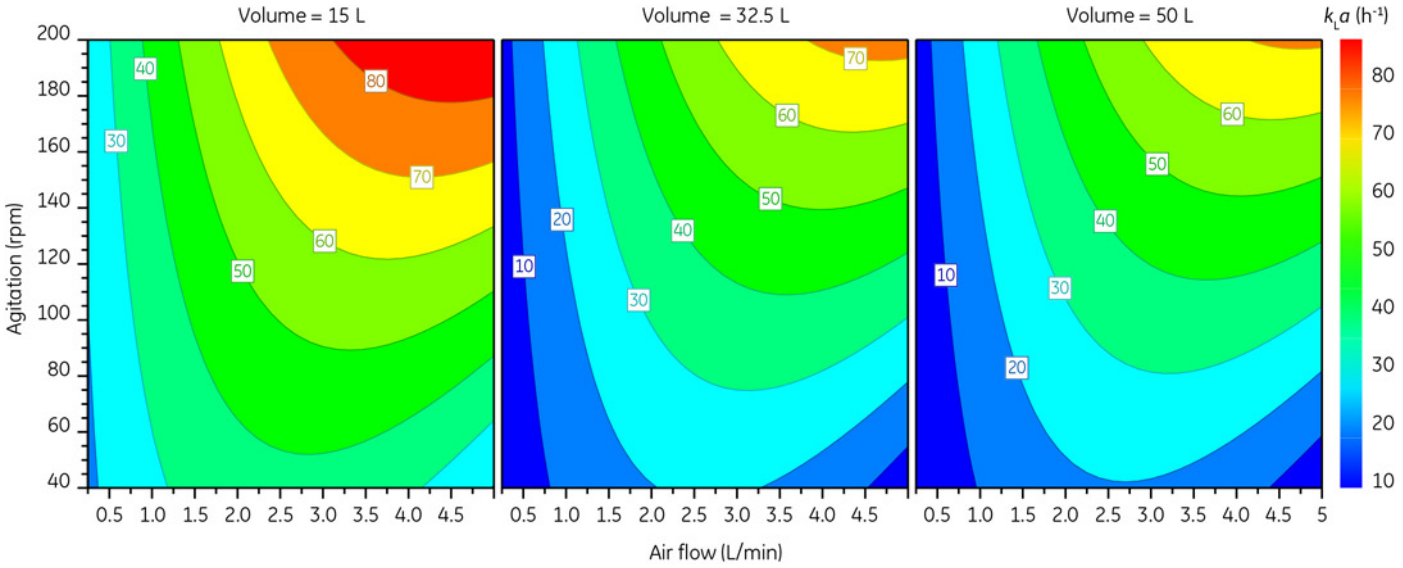

Fig 11. 4D response contour plot displaying k_La obtained with the 2 µm sparger pore size at varying air flow, volume, and agitation. The model fit (R^2) is 0.95, the model predictability (Q^2) is 0.88, and the residual standard deviation (RSD) of the model is 6.37 h^{-1}.

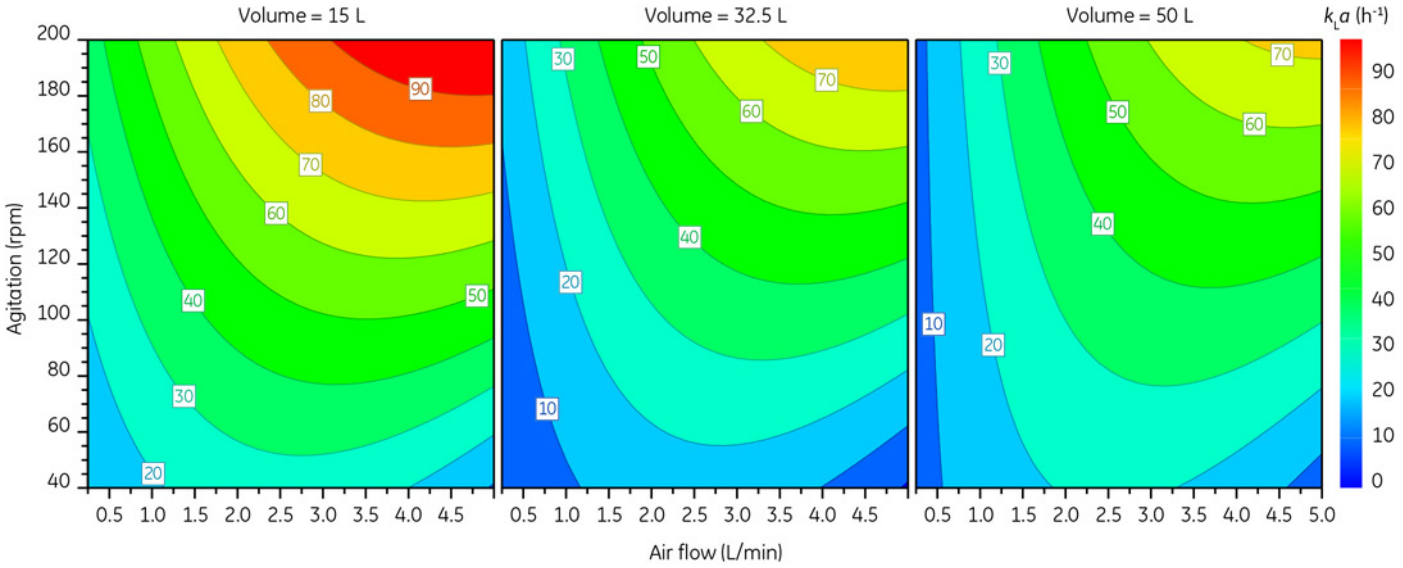

Fig 12. 4D response contour plot displaying k_La obtained with the 20 µm sparger pore size at varying air flow, volume, and agitation. The model fit (R^2) is 0.98, the model predictability (Q^2) is 0.93, and the residual standard deviation (RSD) of the model is 5.28 h^{-1}.

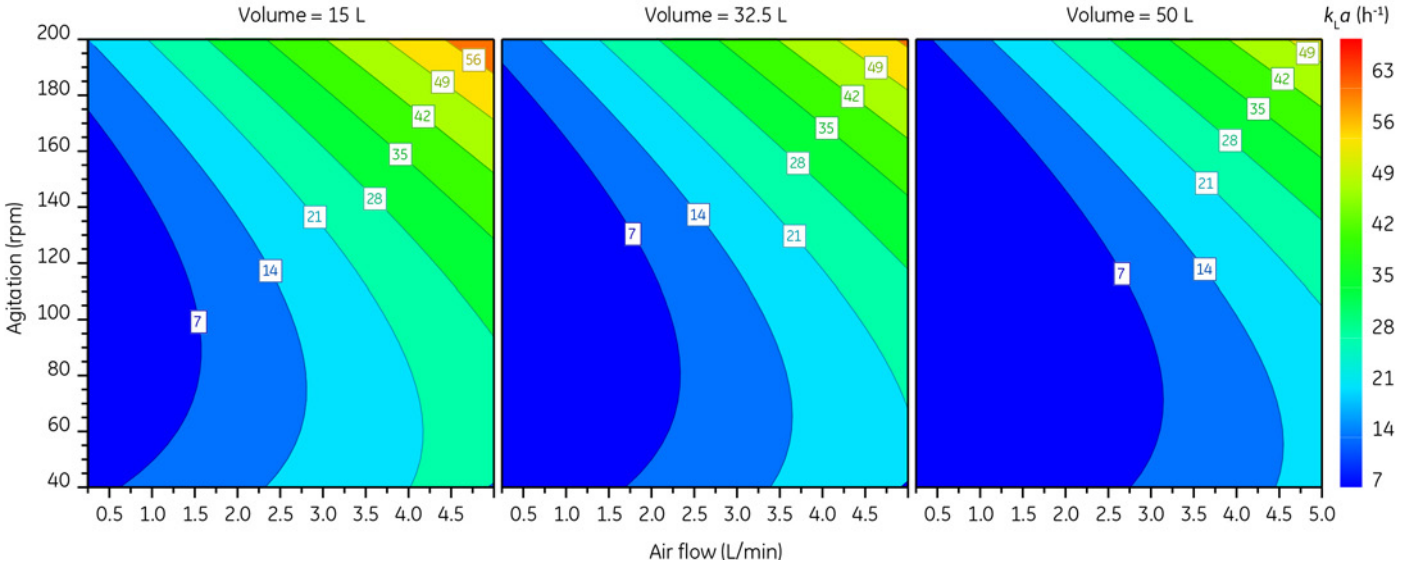

Fig 13. 4D response contour plot displaying k_La obtained with the 0.5 mm sparger pore size at varying air flow, volume, and agitation. The model fit (R^2) is 0.93, the model predictability (Q^2) is 0.87, and the residual standard deviation (RSD) of the model is 5.08 h^{-1}.

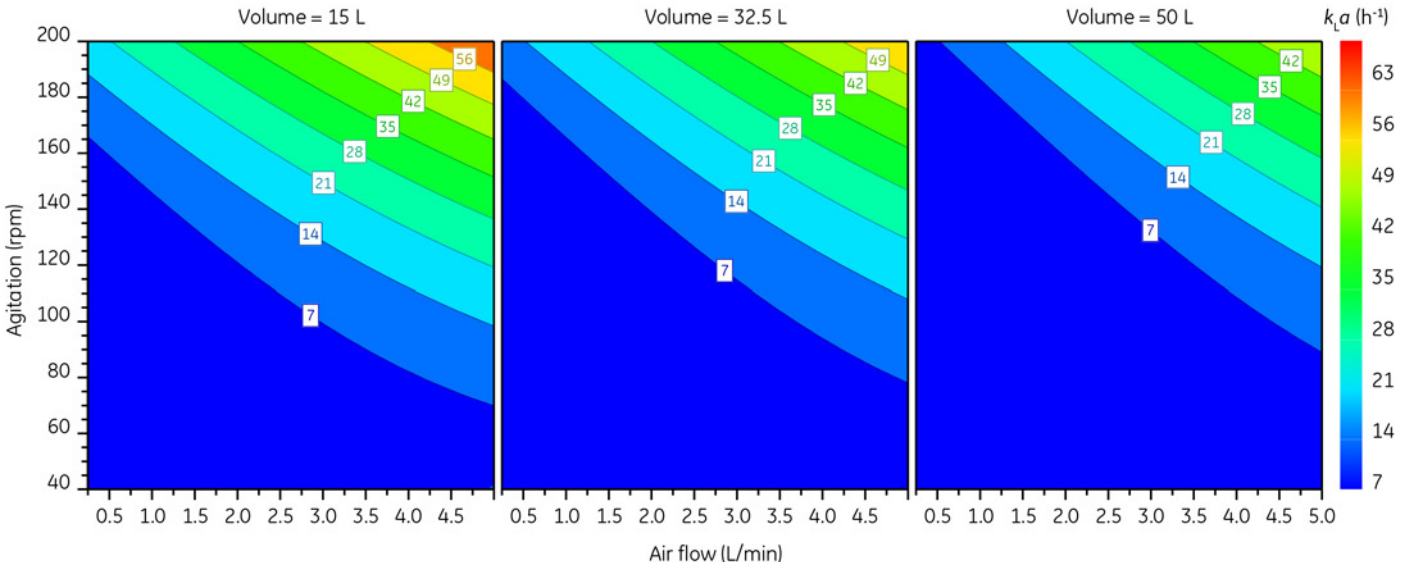

Fig 14. 4D response contour plot displaying k_La obtained with the 1.0 mm sparger pore size at varying air flow, volume, and agitation. The model fit (R^2) is 0.98, the model predictability (Q^2) is 0.92, and the residual standard deviation (RSD) of the model is 3.44 h^{-1}.

Maximum oxygen transfer rate (OTR_{max})

Results from OTR_{max} measurements using the XDA 50 L fermentor bag are shown in Figure 15. The highest OTR_{max} recorded was 1114 mmol/L/h at a 15 L volume in up-flow agitation direction. The results indicate that up-flow agitation direction gives somewhat higher OTR_{max} than down-flow agitation direction.

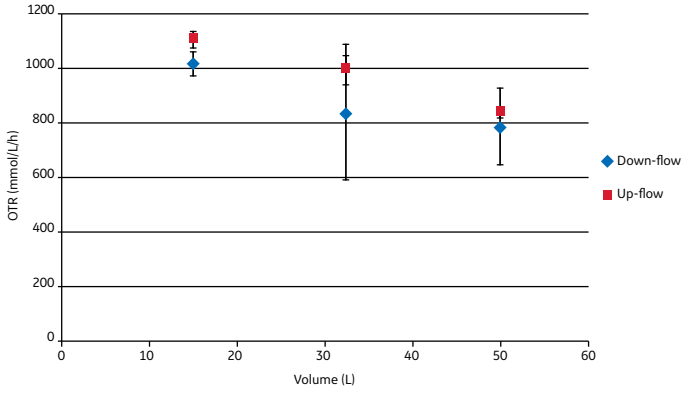

Fig 15. Results from OTR_{max} measurements. Error bars correspond to three standard deviations.

Conclusions

The dual-purpose XDR-50 stirred-tank bioreactor system can be used in both cell culture and microbial fermentation applications. This application note gives a detailed description of the physical characteristics of the system when equipped with either an XDA 50 L cell culture bag assembly or an XDA 50 L fermentor bag assembly. Mixing time results show sufficient mixing capacity to ensure a well-mixed tank. Heating and cooling capability 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. Oxygen transfer rates were found to be well-suited for microbial applications. 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.

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-50 development bioreactor bag	50 L, pitch blade impeller, one disc each of 2 µm, 20 µm, 0.5 mm, and 1 mm spargers and two discs with 0.5 mm orifice diameter	888-0356-C
XDA-50 microbial fermentor bag	50 L, with two-stage impeller with an axial-flow pitched-blade impeller on top of a Rushton impeller and eight open tube spargers with 0.08 inch diameter.	888-0235

To order the XDR-50 system, please contact your local sales representative.

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