Engineering characterization of the single-use Xcellerex XDR-2000 stirred-tank bioreactor system

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Engineering characterization of the single-use Xcellerex™ XDR-2000 stirred-tank bioreactor system

This application note describes the physical characteristics of the XDR-2000 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-2000 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-2000 in terms of mixing time, heating and cooling times, power input, and k_La when equipped with a single-use XDA 2000 L cell culture bag assembly.

Materials and methods

System setup

The XDR-2000 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 2000 L cell culture Pro bag assembly with an integral pitched-blade impeller and eight discs of micro spargers with 20 µm 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 assessed by determining the time required to reach 95% of a pH step change (t_{m95}) (Fig 1). Time zero was selected as the time at which the first sensor reacted to addition of acid in each run. The XDA 2000 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 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: 400, 1200, 2000 L and 25, 70, and 115 rpm, respectively. The temperature was controlled at 37°C and the impeller was run counterclockwise to provide an upward fluid flow.

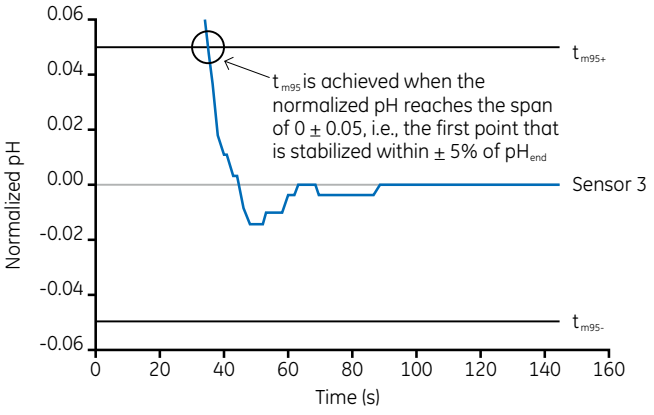

Fig 1. Example of normalized pH data for t_{m95} determination. The black lines indicate the interval where t_{m95} is achieved.

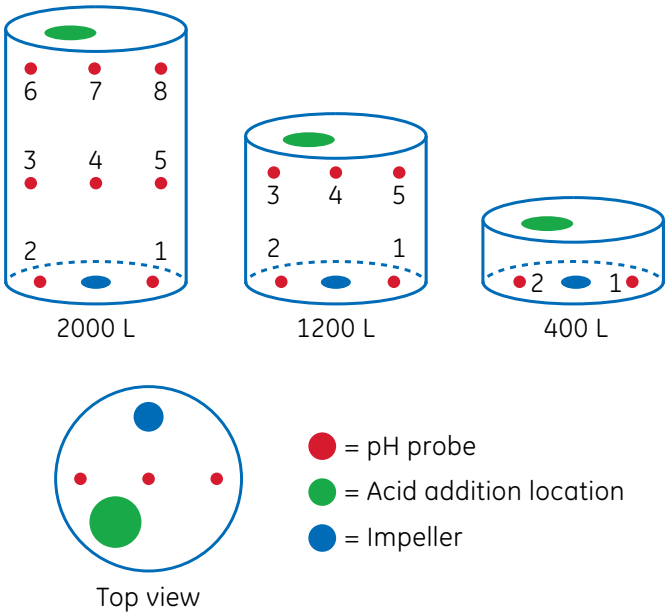

Fig 2. The pH probe positions for the tested volumes. Eight probes in three levels were used to record the pH for 2000 L. At 1200 L, the top probe layer was removed and the pH was recorded by using five probes in two levels. At 400 L, the pH probes of the midsection were removed, leaving the bottom pH probes. 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: 400, 1200, and 2000 L. The XDA 2000 L cell culture bag was filled with a saline solution consisting of 6 g/L of NaCl dissolved in water. The upward fluid flow was held constant at an agitation rate of 75 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-2000 system was assessed using two different methods. In Method 1, the motor current was recorded from the servo drive of the XDR-2000 system and converted into torque using the torque constant 2.04 (± 10%) Nm/A (Kollmorgen, Radford, VA, USA). Method 2 was based on direct torque measurements using a rotary torque sensor incorporated into the shaft. The sensor output was converted to torque using the calibration constant specific for the torque sensor.

The XDA 2000 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 water to a volume of 2000 L. Power input was measured using both methods at agitation rates between 25 and 115 rpm (upward fluid flow) under gassed (20 L/min) and ungassed conditions. Temperature was controlled at 37°C. 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. The following equations provide more detail of the calculations done.

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

Method 1:

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

Method 2:

[Eq. 2]: $P = (\tau_{load} - \tau_{Izero\ load}) * 2\pi N$

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

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

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

[Eq. 4]: $N_p = \frac{N * D_i^2 * \rho}{\mu}$

Power per volume is calculated according to:

[Eq. 5]: $P = P/V$

Where:

P = power input in W

I_{load} = motor current at load conditions in A

$I_{zero\ load}$ = 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)

ρ = medium density in kg/m^3 (here 1000 kg/m^3 was used)

μ = medium viscosity in kg/m/s (here 0.001 kg/m/s was used)

V= volume in m^3

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. 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, and 50 ppm active silicone (Antifoam C, Sigma). Testing was performed at three liquid volumes of 400, 1200, and 2000 L, and the temperature was controlled at 37°C to simulate typical culture conditions. Agitation was varied between 25, 70, and 115 rpm in the up-flow direction. Air flow rate was varied between three levels: 2, 11, and 20 L/min. To measure DO, a standard XDR DO sensor (Hamilton) was used. All eight sparger discs with 20 µm 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_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. 6]: $ln(DO^* - DO_t) = -k_La(t - t_0) + ln(DO^* - DO_{t0})$

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 run is plotted to display the worst-case scenario. The shortest t_{m95} was determined to 21 s when measured at 400 L and 115 rpm, whereas the longest t_{m95} was determined to 199 s when measured at 2000 L and 25 rpm. Figure 4 shows the t_{m95} for each probe position in experiments performed at maximum working volume (2000 L) viewing how the mixing time varied 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 37 s, indicating effective mixing across the whole bioreactor.

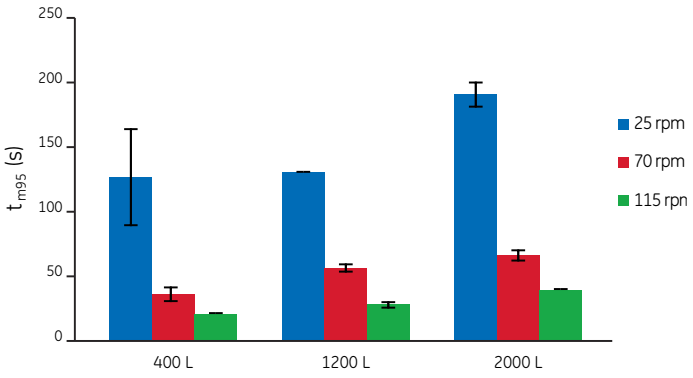

Fig 3. Average mixing times (t_{m95}) from the probe positions that generated the longest mixing time for all tested volumes and agitation rates. Error bars correspond to one standard deviation.

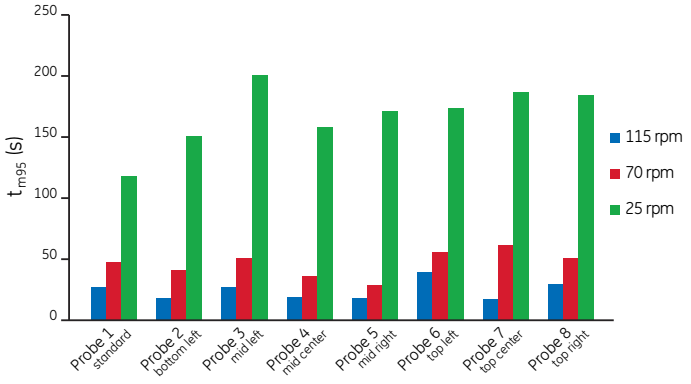

Fig 4. Mixing time (t_{m95}) for each probe position for maximum working volume (2000 L), tested at low, mid-point, and 115 rpm. Results are averaged values from duplicate runs.

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 5.1 h for all volumes tested. Cooling the liquid from 37°C to 5°C required the longest time, between 7 and 21 h depending on the volume. In general, the time required for heating and cooling was found to increase with liquid volume.

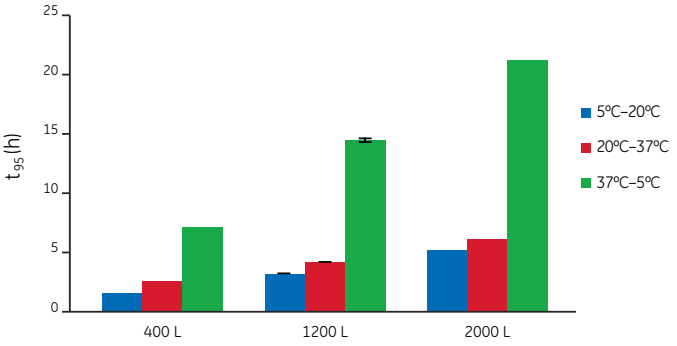

Fig 5. Results from the heating-cooling experiments. Error bars correspond to one standard deviation.

Power input

Figure 6 shows volumetric power input (P/V) at maximum working volume (2000 L) plotted against agitation rate for gassed (20 L/min) and ungassed conditions using Method 1 and 2. The difference in power input between ungassed and gassed conditions was negligible. Comparing Method 1 (motor current) with Method 2 (torque sensor) shows that the power input obtained using the torque sensor was somewhat higher than the results obtained by the motor current.

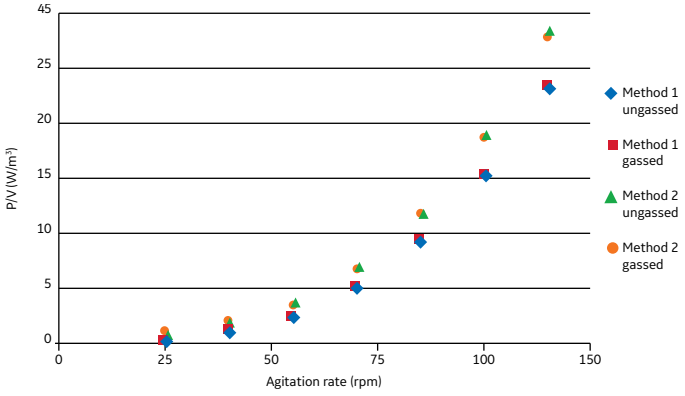

Fig 6. Power input per liquid volume (P/V) plotted against agitation rate at max. working volume (2000 L) for Method 1 (motor current) and Method 2 (torque sensor) at gassed and ungassed conditions. The air flow was 20 L/min for gassed conditions. Each data point shows the mean value of duplicate runs.

Figure 7 shows power number, calculated from the ungassed conditions, plotted against agitation rate and Reynolds number for the two different methods.

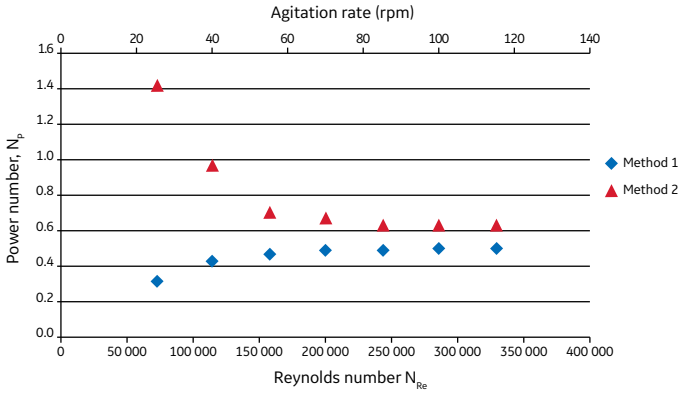

Fig 7. Power number (N_P) plotted against agitation rate and Reynolds number (N_{Re}) calculated from the ungassed power measurements.

Volumetric oxygen transfer coefficient (k_La)

Results from k_La determination are shown in Figure 8 (non-modelled k_La data plotted in column charts) and Figure 9 (contour plot generated by the DoE software from experimental data). A model with good fit, reproducibility, and predictability was obtained. Measured k_La values ranged from 1.1 to 19.7 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. The average response time (t_{63}) of the DO probes used was 26 s, which should be sufficient for determining k_La values of acceptable accuracy up to 139 h^{-1} in accordance with $t_{63,\ crit} = 1/k_La_{max}$ (1).

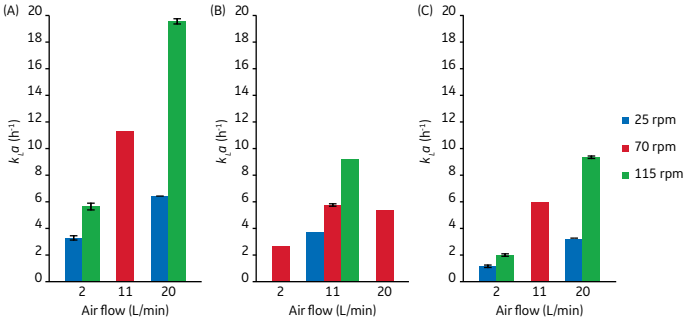

Fig 8. Results from the k_La experiments with the 20 µm sparger pore size in (A) 400 L, (B) 1200 L, and (C) 2000 L. The DoE corner points were run in duplicates and the center point was replicated in triplicate runs. The error bars correspond to one standard deviation.

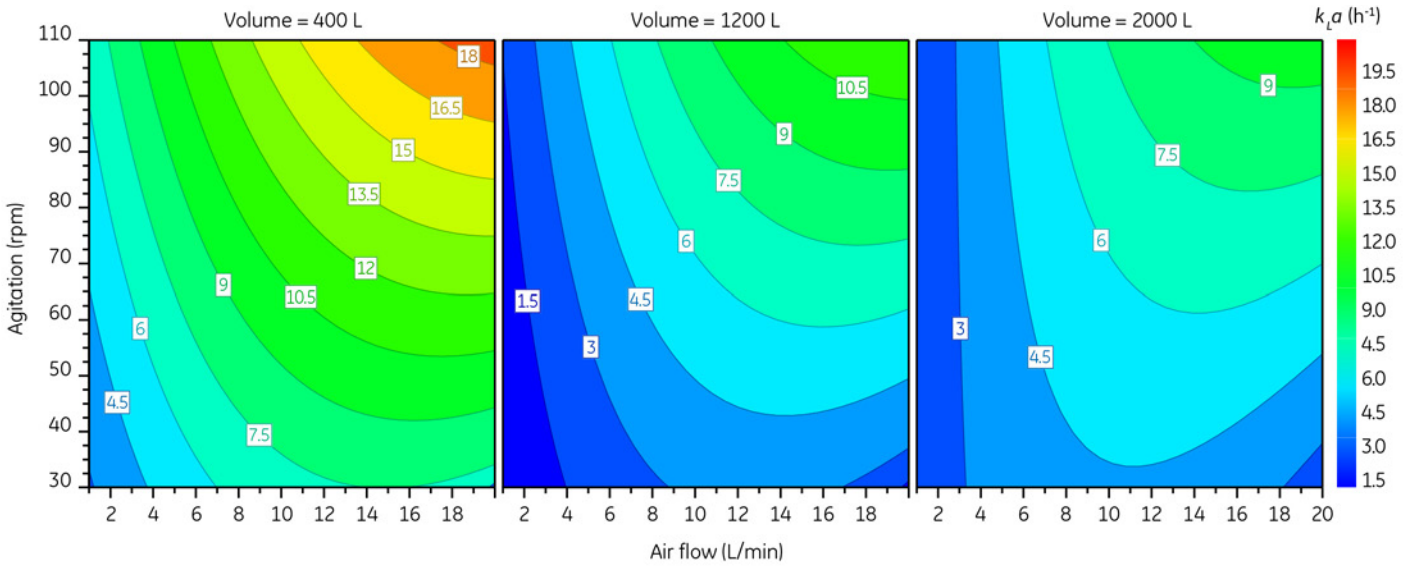

Fig 9. 4D response contour plot displaying k_La obtained with the 20 µm sparger pore size at varying air flow, volume, and agitation. The model fit (R^2) is 0.97, the model predictability (Q^2) is 0.93, and the residual standard deviation (RSD) of the model is 0.98 h^{-1}.

Conclusions

This application note gives a detailed description of the physical characteristics of the XDR-2000 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.

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-2000 Pro bioreactor bag	2000 L, pitch blade impeller, and eight discs of micro spargers (20 µm)	888-0081-C

To order the XDR-2000 system, please contact your local sales representative.

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