Performance of single-use Xcellerex XDR-500 MO and XDR-50 MO stirred-tank fermentor systems

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Performance of single-use Xcellerex™ XDR-500 MO and XDR-50 MO stirred-tank fermentor systems

This application note describes the performance of the XDR-500 MO fermentor system in comparison with the XDR-50 MO fermentor. Domain antibody (dAb) production in *E. coli* was used as the model process. Fermentations were performed as fed-batch processes, with glycerol feed and a two-stage induction strategy. The presented data was collected as foundational single-use fermentation work, and can be used when scaling between the XDR-50 MO and XDR-500 MO systems.

Introduction

The process design proven for one fermentor system is not always directly transferrable to other system formats without adaptation. Hence, it is necessary to understand the interrelation between the physical features of a fermentor and how these features ultimately affect the performance of the system.

Here, we compare the performance of the XDR-500 MO fermentor with the XDR-50 MO system. A process for dAb production in *E. coli*, initially developed for a conventional stainless steel 20 L fermentor, was used as the model. The described process was scaled from a XDR-50 MO fermentor culture, with a start volume of 30 L, to a larger XDR-500 MO fermentor culture, with a start volume of 350 L. The process was adapted to, but not optimized for, the 50 and 500 L pair of single-use fermentor systems.

Materials and methods

Medium preparation

Culture medium was prepared as described (1). Medium components, except those that are precipitation-prone, were premixed in single-use Xcellerex 500 and 100 L mixing systems for XDR-500 MO and XDR-50 MO, respectively. The mixtures were sterile-filtered (0.2 µm) into the single-use fermentor bags prior to culture start. Components that are precipitation-prone or difficult to filter (trace elements, $MgSO_4$, kanamycin, and antifoam) were added separately as sterile solutions directly into the single-use fermentor bag prior to inoculation.

Organism

Production of dAb from the *E. coli* RV308 strain, with a pBR322-derived plasmid coding for the dAb as well as for kanamycin antibiotic resistance, was used as the model process. The dAb production is under the control of an inducible promoter, and the protein is secreted into the periplasm. Seed cultures were prepared as described earlier (1).

Fermentor preparation

Fermentations were performed with the single-use XDR-50 MO (using 30 L culture volume at start) and XDR-500 MO (using 350 L culture volume at start) systems. The systems were prepared according to standard procedures as previously described (1). Gamma-irradiated XDA fermentor bags (50 and 500 L for XDR-50 MO and XDR-500 MO, respectively), including fermentation-specific impeller assemblies, were installed. The single-use fermentors were further equipped with condenser bags and exhaust gas filters. These accessories are integrated with the XDA 50 L MO bag, while installed separately with the XDA 500 L MO bag. Both single-use fermentors were equipped with autoclaved dissolved oxygen (DO) and pH probes.

Fermentation process

The process parameters at inoculation are summarized in Table 1. DO level of the cultures was kept at set point with the help of a cascade control of agitation, airflow, and O_2 flow. To keep DO set point, the controller progressively increased the agitation rate up to its maximum level, after which O_2 enrichment of airflow was initiated (maximum allowed mixing 50%:50% air-to-O_2). Two aeration strategies were applied. In XDR-500 MO, air flow rate was constant with increasing O_2 gas flow rate, while in XDR-50 MO, total gas flow was maintained constant. Temperature control was performed with separate temperature control units (TCU) from PolyScience (model no. 58903TD7XC751) and Mokon (model no. 311394-10CE) for XDR-50 MO and XDR-500 MO, respectively.

The feed profiles for all cultures are shown in Figure 1. Maximum feed rate in XDR-500 MO runs was decreased after each run to improve DO control via O_2 enrichment and substrate utilization by the cells. Expression of dAb was triggered with a two-step induction strategy: a first induction was performed by addition of isopropyl ß-D-1-thiogalactopyranoside (IPTG) to the culture, followed by a second IPTG addition to the same final concentration 3 h later. Prior to the first induction event, a shift in cultivation temperature from 37°C to 30°C was applied. The fermentations were terminated after approximately 20 to 26 h total run time. After the fermentation, the cultures were heat treated at 48°C for 3 h to release the produced dAb from the cell periplasm into the culture medium.

Table 1. Summary of key process parameters

Parameter		XDR-50 MO (run A, B)	XDR-500 MO (run A, B, C)
Process set point	Temperature	37°C	37°C
	pH	7.2	7.2
	DO	30%	30%
	Agitation	250 rpm	**A:** 250 rpm **B, C:** 200 rpm
	Aeration*	15 L/min air, 0 L/min O_2	250 L/min air, 0 L/min O_2
Inoculation	Target OD_{600}	0.01	0.1
Induction	IPTG	2 x 0.5 mM	2 x 0.5 mM

*Initial set point that changes over the course of the process.

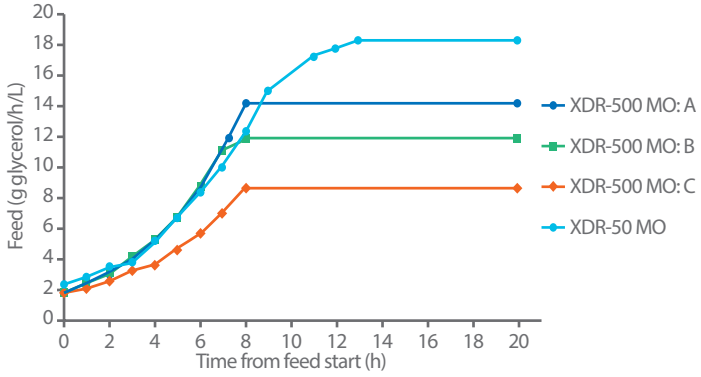

Fig 1. Feed profiles of the fermentation runs.

Analysis

Concentration of expressed dAb was determined by protein-L based affinity chromatography using Capto™ L resin packed in a HiTrap™ column.

Results

Fermentation

The optical density (OD) values for the different cultures are shown in Figure 2. By reducing the substrate feed rate in the XDR-50 MO cultures, DO control was improved (O_2 enrichment stopped well below its maximum 50% value), while *E. coli* growth was not affected. Final OD ranged from 105 to 153 (average 129) for the XDR-50 MO cultures, and from 117 to 144 (average 129) for the XDR-500 MO cultures. Final OD values are shown in Figure 3.

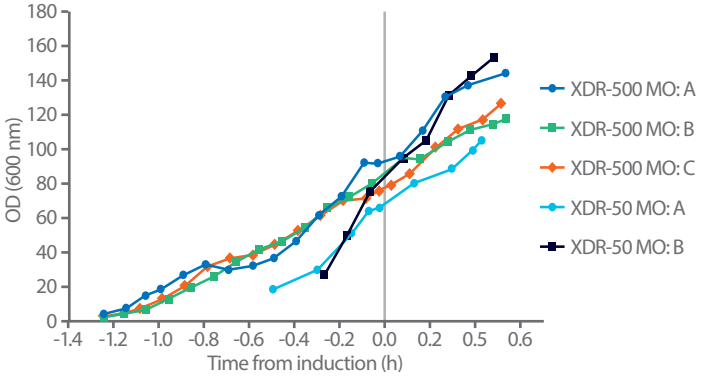

Fig 2. OD plotted against cultivation time normalized against induction. Time 0 h = first induction. XDR-50 MO cultures were not sampled for OD during the first hours of fermentation.

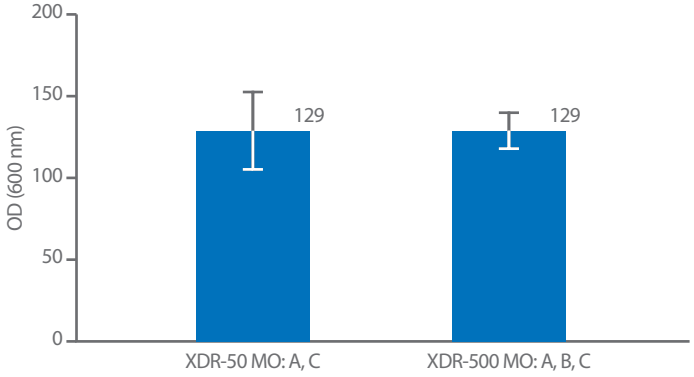

Fig 3. Final OD values. Average process duration was 20 h.

Temperature change step

At induction, the temperature was decreased from 37°C to 30°C. An equivalent cooling performance was observed between the XDR-50 MO and XDR-500 MO systems. In both single-use systems, an induction temperature of 30°C was reached within 0.5 h, with an average cooling rate of 17°C/h in XDR-50 MO and 27°C/h in XDR-500 MO. Maximum cooling rate in XDR-500 MO was 41°C/h.

When the process was finished, the cultures were heat treated at 48°C for 3 h to release produced dAb from the *E. coli* periplasm into the culture medium. For both systems, the temperature set point 48°C was reached within 1.5 h, at a maximum rate of 20°C/h. Also for heating, an equivalent performance was observed between the fermentor systems.

Process control

Evaluation of process control in XDR-50 MO and XDR-500 MO fermentations is shown in Figure 4. All curves were plotted against cultivation time, with zero being the time point for inoculation in all runs.

In XDR-500 MO, an aeration strategy with an initial low gas flow rate was used to avoid foaming in the beginning of the culture period. A constant air flow of 250 L/min was used with continuously increasing O_2 gas flow, that is, total gas flow rate was continuously increased from initial 250 to 420 L/min.

In the XDR-50 MO runs, on the other hand, total gas flow was initially 15 L/min (only air). When agitation had been increased to its maximum level, set point for air flow was gradually increased to 30 L/min, after which the total gas flow was maintained constant at 30 L/min with varying air/O_2 composition.

Although the applied strategies for oxygenation with O_2 and air gas flow were different in XDR-50 MO and XDR-500 MO, DO control was comparable between the systems, indicating that similar parameter control can be achieved with the systems independent of size.

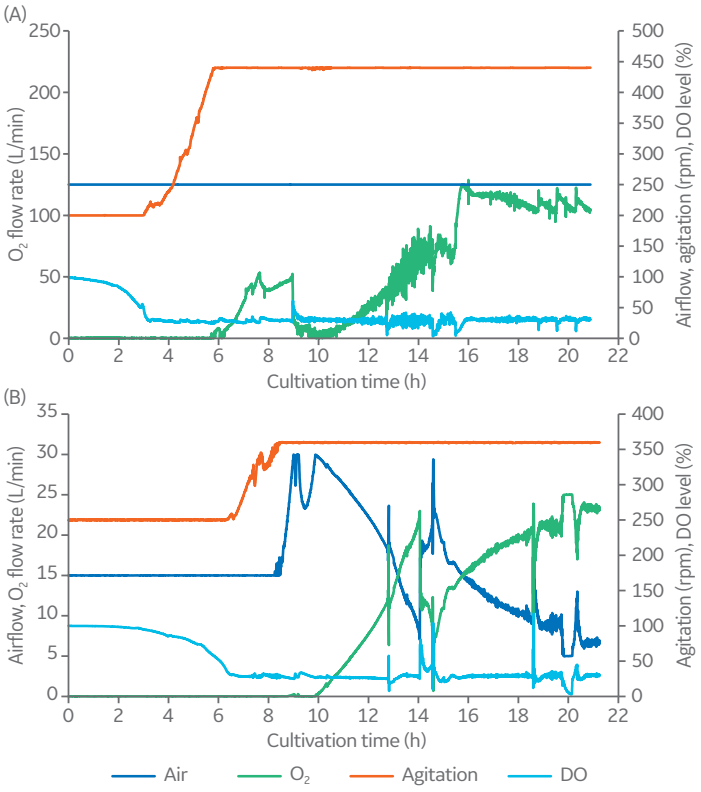

Fig 4. Example of DO curves for (A) XDR-500 MO and (B) XDR-50 MO fermentations plotted against cultivation time.

Conclusion

Results show that the XDR-500 MO fermentor has sufficient capacity to enable equal performance to XDR-50 MO with regard to oxygenation and temperature control throughout a fed-batch fermentation process. Additionally, the ability to achieve similar OD values indicates that the same protein production level is possible with both fermentor systems. In this work, the basis for the scale-up was to find conditions for achieving similar oxygen transfer in XDR-500 as in XDR-50. Based on the ability to maintain critical process parameters such as DO, temperature, and pH, the presented work demonstrates predictable scaling between the XDR-50 MO and XDR-500 MO fermentor systems. The work also shows that a fed-batch fermentation process can be successfully transferred from a conventional stainless steel fermentor to the single-use XDR-50 MO and XDR-500 MO fermentors, and that comparable results can be expected without the need for extensive process adaptations. The results support use of the XDR fermentors as seed train or production bioreactors, allowing a flexible setup of fermentors in different scales and operating environments.

Reference

1. Application note: *E. coli* growth and domain antibody (dAb) expression in single-use and stainless steel fermentors. GE Healthcare, 29134111, Edition AA (2015).

Ordering information

Product	Description/size	Product code
888-0235	XDA 50 L microbial fermentor bag, including a two-stage impeller, with an axial-flow (pitched-blade) impeller on top of a Rushton impeller, and eight open tube spargers with a diameter of 0.08 inch. Condenser bag and exhaust gas filter are integrated.	29041537
888-2-0713-C	XDA 500 L microbial fermentor bag, including a multi-stage impeller comprising two axial flow (pitch-blade) and three Rushton (six-blade) impellers, and a 28 hole disc sparger with diameter of 0.125 inch.	29087672
888-2-0714	XDA condenser bag, part of the XDR-500 MO consumable set.	29087673
888-2-0715	XDA exhaust filter tube set (20 inch), part of the XDR-500 MO consumable set	29087674
888-0138	XDR probe sheath assembly for DO and pH probes.	29041158

To order the XDR-500 MO and XDR-50 MO fermentor systems, please contact your local sales representative.

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