5-7-2017

# XDR-500 MO—Single-use fermentor for microbial processes

Jozsef Vasi
*GE Healthcare, Sweden*

Andreas Castan
*GE Healthcare, Sweden*, andreas.castan@ge.com

Sandeep Kristiansson
*GE Healthcare, Sweden*

# Xcellerex™ XDR-500 MO single-use fermentor for microbial processes

*József Vasi, Andreas Castan and Sandeep Kristiansson*

*GE Healthcare Bio-Sciences AB, Björkgatan 30, 75184 Uppsala, Sweden*

## Abstract and introduction

Single-use bioreactors are well-established in mammalian cell-based biopharmaceutical productions. However, adoption of single-use technologies for microbial/yeast-based production has been slow due to the unique requirements of fermentation processes. The typical challenges include supporting high-cell density cultivations, high oxygen transfer capacity, and efficient heat removal.

Here, we present the single-use XDR-500 MO stirred-tank fermentor system specifically designed for microbial/yeast applications. The performance of the fermentor was evaluated using an *E. coli* fed-batch process producing a domain antibody (dAb) as a model system. The process was originally developed for a lab-scale conventional stainless steel (SS) system. This work describes the strategy applied to transfer and scale-up this process to the single-use bioreactor (SUB) without the need for extensive re-optimization due to technology change. Moreover, process economy of dAb production based on single-use and conventional technology was compared *in silico.*

Cell densities and dAb expression levels similar to the lab-scale conventional bioreactor were achieved in XDR-500 MO, confirming feasibility of single-use technology even in microbial processes.

## Materials and methods

Fed-batch process for *E. coli* RV308 clone expressing a dAb, originally developed for lab-scale conventional SS bioreactor, was transferred and scaled up to XDR-500 MO SUB. Mineral salt medium complemented with yeast extract in combination with 60% (w/v) glycerol substrate feed was used for cultivations. Process parameters critical for dAb expression were kept constant between technologies, while others were adapted to single-use technology (Table 1). Dissolved oxygen level was cascade controlled by stirrer speed, airflow, and oxygen enrichment of airflow in the single-use system.

Substrate feeding strategy was adapted to the SUB and three different rates were evaluated.

Medium and feed solutions were prepared in Xcellerex XDUO mixer bags and sterile filtered into the to single-use bioreactor. All cultures have been induced at an $OD_{600nm}$ of 80.

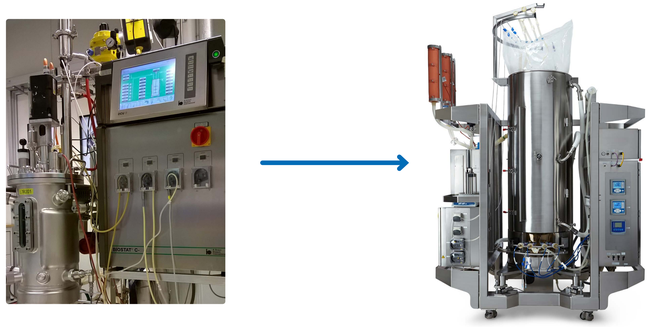

**Table 1**. Process transfer and scale-up strategy

**Process parameters**

| Kept constant | Adjusted |
|---|---|
| pH and DO set points | DO control strategy |
| Medium and feed composition | Medium and feed preparation procedures |
| Induction conditions | Substrate feed profile |
| Temperature set point | |

## Experimental results

Improved process understanding during transition to SUB indicated substrate overfeeding in the SS bioreactor. Evaluation of three reduced feed regimes showed that even applying as low as 60% of the original feed rate to XDR-500 MO was sufficient to maintain cell growth, as shown for XDR-Run 3 in Figure 1, and product formation was found to be at comparable levels to the original process. The reduced substrate feed also led to decreased oxygen consumption rate.

Levels of expressed dAb achieved in XDR-500 MO after process transfer and scale-up were reproducible and comparable with the SS bioreactor (Fig 2) even without process optimization. Furthermore, it was shown that dAb concentration increased linearly after induction (Fig 3), indicating a straightforward strategy to improve product titer.

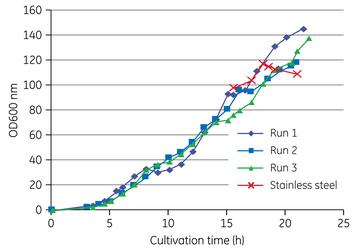


**Fig 1.** Cell growth in XDR-500 MO at studied feed rates. Cell growth even at lowest feed rate was comparable with stainless steel bioreactor.

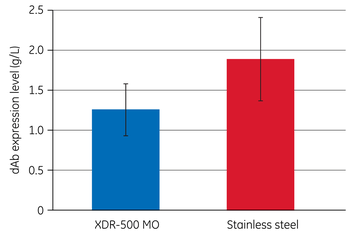


**Fig 2.** Comparison dAb expression levels obtained in single-use XDR-500 MO and stainless steel reactors. Product concentrations were determined after 5 h induction time.

Critical process parameters like dissolved oxygen level, pH, and temperature were controlled efficiently within their targeted range under the entire cultivation. Temperature was maintained at 37˚C ± 0.1˚C even when metabolic heat generation peaked at 38 000 BTU/h. Instead of increasing headspace pressure, DO was maintained with oxygen enrichment, enabling cell growth to high cell densities (Fig 4).

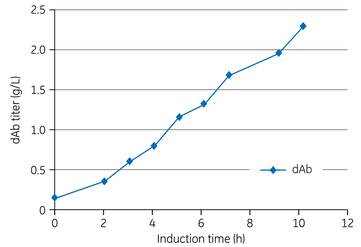


**Fig 3.** Effect of extended induction time on dAb expression.

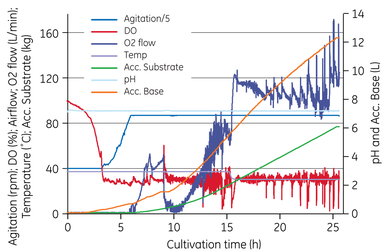


**Fig 4.** Parameter chart of a typical dAb cultivation in the single-use XDR-500 MO.

## Process economy

Comparison of process economy between single-use and conventional SS production facilities for dAb manufacturing revealed up to twice as high facility utilization when producing in a single-use system (Table 2).

According to our model, at 4–15 annual batches (e.g., for toxicology or early clinical phase studies), it is more cost-effective to manufacture in a single-use facility compare with a conventional facility (Fig 5).

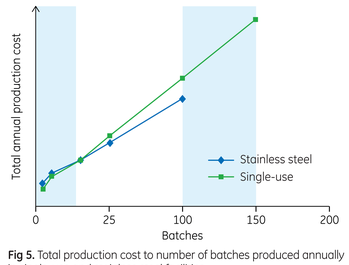


**Fig 5.** Total production cost to number of batches produced annually in single-use and stainless steel facilities.

**Table 2.** Comparison of process economy for dAb production between XDR-500 MO SUB and SS bioreactor (scenarios for single and multiple product facilities were evaluated)

| Parameter | Scenario 1 – Single product facility | | Scenario 2 – Multi product facility | |
|---|---|---|---|---|
| Type of Bioreactor | SS | SUB | SS | SUB |
| Maximum number of batches | 100 | 150 | 67 | 135 |
| Relative cost comparison | 1× | 1.47× | 1× | 1.40× |
| Relative facility utilization | 1× | 1.5× | 1× | 2× |

## Conclusions

- XDR-500 MO allowed robust control of challenging process parameters (DO, pH, temperature).
- The model process successfully transferred and scaled up from SS bioreactor to SUB without the need for extensive modifications.
- Cell growth and dAb production level were comparable across technologies/scales even with the implemented changes.
- Higher production flexibility and facility utilization can be achieved with single-use systems.
- Single-use systems are viable alternatives to conventional SS bioreactors for microbial processes.


29254432 AA 03/2017