GE Healthcare
Life Sciences

Microbial fermentation in single-use Xcellerex™ XDR-50 MO fermentor system

This application note describes the performance of a single-use Xcellerex XDR-50 bioreactor system when used in cultivations of *E. coli* and of modified *Pseudomonas fluorecsens* (*P. fluorescens*) producing a monoclonal antibody (MAb). Both the achieved microbe densities and product yield were shown to be consistent with the performance of conventional stainless steel systems.

Introduction

The adoption of single-use technology by biomanufacturers using microbial systems has been inhibited by the lack of single-use bioreactor systems capable of accommodating the unique requirements of microbial cultures. Bioreactors designed for production in mammalian culture fall short of meeting these unique requirements, for example in terms of oxygen transfer capacity and temperature control. The Xcellerex XDR-50 MO fermentor system is purpose-designed and built to overcome the mammalian single-use system limitations in fulfilling these needs (Fig 1).

The XDR-50 MO fermentor is part of Xcellerex scalable, single-use bioreactor family for working volumes ranging from 4.5 L to 2000 L. The system design of XDR stirred-tank bioreactors enables easy scaling and facilitates the achievement of a robust, final manufacturing-scale process without significant process modification.

The XDR-50 MO single-use fermentor is a 50 L turnkey, modular system that delivers performance comparable with hard-piped, stainless steel fermentor systems. Single-use technology eliminates time-consuming and costly clean-in-place (CIP), steam-in-place (SIP), and cleaning validation procedures. The system's turnkey design enables fast installation and start-up as well as rapid batch-to-batch turnover along with increased process flexibility compared with fixed, stainless steel, hard-piped vessels. Each XDR-50 MO fermentor system includes a stainless steel, jacketed vessel, vital process instrumentation, robust automation, and an optimized, single-use bioreactor bag assembly. The system only requires gases and an electric supply to be fully operational.

Fig 1. XDR-50 MO fermentor vessel and I/O cabinet.

The vessel features a dimpled jacket heat transfer surface for efficient cooling and heating, a high performance bottom-mounted, magnetically coupled drive, loadcells for weight measurement, an exhaust filter heater, and removable baffles.

Process instrumentation includes mass flow controllers, peristaltic pumps, and probe transmitters. An external temperature control unit is available as an option.

The XDR-50 MO fermentor is equipped with a stand-alone, movable control console, featuring intuitive process controls, data historian, and industrial-quality automation hardware and software. The system provides real-time data acquisition, enables accurate process control, and offers convenient, real-time trending.

The heart of the XDR-50 MO fermentor system is the XDR single-use bag assembly, designed and built to meet the stringent requirements of microbial fermentation. The single-use bag assembly is based on the design and materials used in the proven XDR technology for mammalian cell culture. The bag assembly consists of a USP Class VI low-density polyethylene fluid contact layer, tubing for liquid addition and harvest, gas sparging plate, sampling and probe ports, pressure sensor, filtered gas lines, and a high-power input agitator system. The exhaust line is fitted with a condenser to prevent exhaust filter blockage. The sparging plate has eight ¼" open pipe ports providing the high gassing rates required to maintain the dissolved oxygen (DO) necessary for microbial cultures. Robust agitation is provided by a powerful magnetic drive and a two-stage impeller (Fig 2). The first stage is a Rushton impeller which delivers excellent power into the system. The second stage is an axial-flow impeller operating in a pump down mode, improving gas residence time. The two-stage impeller combination results in very high oxygen transfer (> 1000 mM/h) to the culture medium.

Fig 2. Two-stage impeller for efficient and application-specific performance.

Materials and methods

E. coli *culture*

E. coli (BLR-DE3) was recovered from frozen storage and expanded in shake-flask culture until the cell density was sufficient to inoculate a fermentor culture. The fermentor was filled to 27 L with culture medium and inoculated with 3 L of exponentially growing culture.

Controller set points were pH 7.0, temperature 37°C, and DO 40% (controlled by air/O_2 cascade). The culture was fed by continuous delivery of 40% glucose solution at a rate to maintain a glucose concentration of 1 to 3g/L.

The culture density (OD_{650}) was measured at time 0 and hourly through 24 hours. At 24 hours post-inoculum, the culture reached a peak OD_{650} of approximately 120.

The cultivation was performed in three parallel experiments.

P. fluorescens *culture*

A vial of a modified *P. fluorescens* strain producing a MAb was recovered from frozen storage and expanded in shake-flask culture until the cell density was sufficient (≥ 6 OD_{575}) to inoculate a fermentor culture. The fermentor was filled to 27 L with culture medium and inoculated with 3 L of exponentially growing culture.

Controller set points were pH 6.5, temperature 32°C, and DO 15% (controlled by agitation, air sparge, and O_2 supplementation).

The culture density (OD_{575}) was measured at time 0 and hourly thereafter. When the culture density reached OD_{575} 75 to 85 the temperature set point was reduced to 28.5°C and pH increased to 6.85. The culture was induced by the addition of isopropyl β-D-thiogalactoside (IPTG) to a final concentration of 105 mg/L. Sampling was continued through 48 h.

The culture OD_{575} ranged from 193 to 375 and the MAb yield from 46 to 72 mg/L, as determined by a protein A biosensor (Pall ForteBio™ Corp, Menlo Park, CA).

The cultivation and protein expression were performed in six parallel experiments.

Result and discussion

The single-use XDR-50 MO fermentor system was used to grow cultures of *E. coli* and *P. fluorescens*.

The *E. coli* cultures reached a peak OD_{650} of approximately 120 at 24 h postinoculum. The results from three parallel experiments are presented in Figure 3.

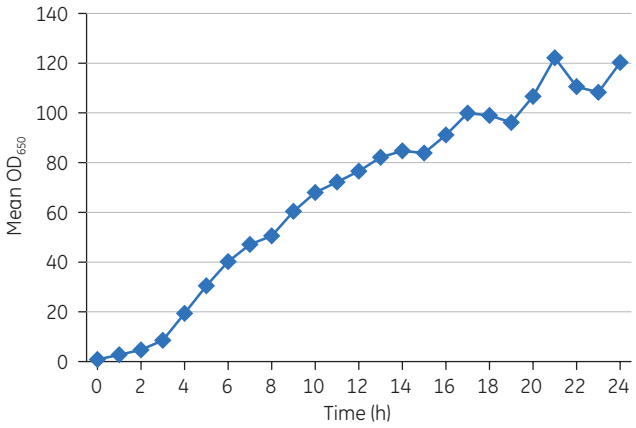

Fig 3. Culture density of *E. coli* grown in XDR-50 MO fermentor system. The figure shows the mean of three parallel runs.

Table 1. Cell and MAb product yields of six representative runs in the XDR-50 bioreactor system

Run No	Culture density (OD_{575})	Dry cell weight (g/L)	OD_{575}/dry cell weight	Packed cell volume (%)	MAb (mg/L)
1	352	131	2.68	45.0	NA
2	223	168	1.33	30.0	46.0
3	282	137	2.05	40.0	55.0
4	193	174	1.1	40.0	68.0
5	242	147	1.64	25.0	72.0
6	375	152	2.47	45.0	56.0
Average	278	152	1.90	37.5	59.4

Modified *P. fluorescens* was grown to culture densities of OD_{575} 75 to 85, when the cultures were induced to produce MAb. The final OD_{575} of the cultures ranged from 193 to 375 and the MAb yields from 46 to 72 mg/L. Both the achieved culture densities and MAb yields are consistent with the expectation for conventional stainless steel systems (1).

Data from six representative runs are presented in Figure 4 and Table 1.

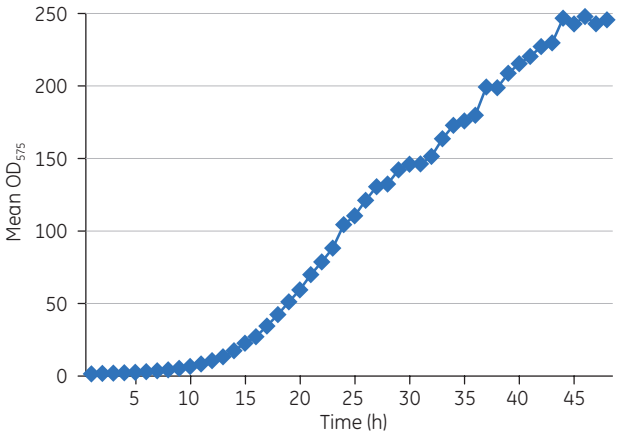

Fig 4. Culture density of *P. fluorescens* grown in XDR-50 MO fermentor system. The figure shows the mean of six parallel runs.

Conclusions

Cultures of *E. coli* and *P. fluorescens* were successfully grown at 30 L scale in a single-use Xcellerex XDR-50 MO bioreactor system, which is specifically designed for microbial fermentation. Both the achieved culture densities and MAb yields were consistent with the expectations.

The fermentor delivers both cell growth and protein production equivalent to those of conventional stainless steel microbial fermentors, but with the improved features and benefits of a single-use system, eliminating the need for time-consuming CIP and SIP operations.

The modular design supports measurement and control of up to six fermentors with one single controller, as well as integration into legacy automation systems.

The adoption of single-use technology by biomanufacturers using microbial systems has been inhibited by the lack of systems capable of accommodating the unique requirements of microbial cultures. The Xcellerex XDR-50 MO fermentor system is purpose-designed and built to fulfill these needs and overcome the previous limitations. The resulting XDR-50 MO fermentor is a powerful, modular turnkey system suitable for use in a GMP production environment.

Reference

Galliher P. Achieving High-Efficiency Production with Microbial Technology in a Single-Use Bioreactor Platform. *BioProcess Int*. **6:**60-65 (2008)

Ordering information

Product	Description	Code number
XDR-50 MO Complete	Single pH, single DO, 50 L	603-F114-E2
XDR-50 MO Complete	Redundant pH, redundant DO, 50 L	603E-F224-E2

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