APPLICATION NOTE

No. COL02036

Scale-up of microbial fermentation using recombinant *E. coli* in HyPerforma 30 L and 300 L Single-Use Fermentors

Jason Brown, Paula Decaria, Nephi Jones, and Tommy Smith

Introduction

Fermentation production facilities can utilize the Thermo Scientific™ HyPerforma™ Single-Use Fermentor (S.U.F.) instead of traditional stainless steel cleaning-in-place/sterilization-in-place (CIP/SIP) fermentor vessels without modifying their existing procedures. As expression systems are moved to production volumes, they can easily be scaled from a benchtop fermentor (or flask) to the S.U.F. Typically, with other single-use bioreactors used as fermentors, culture procedures have to be modified substantially to minimize the risk of negatively affecting the organism or plasmid. The HyPerforma S.U.F. (Figure 1) was designed to meet the unique requirements of microbial fermentation instead of being modified from a cell culture bioreactor.

Figure 1. HyPerforma 30 L and 300 L Single-use Fermentors with condensers.

The S.U.F. utilizes three Rushton-type impellers along with vessel geometric proportions, spacing, baffle configurations, and agitation rates common in rigid-walled industry CIP/SIP fermentors and research fermentors (Figure 2). The motor and direct drive system of the vertically centered drive agitator has been designed and tested to help ensure reliability. The proprietary single-use, sterile, rotary seal-bearing port technology has been proven to be the most reliable and widely used in the biotechnology industry and requires zero maintenance, unlike traditional mechanical seals.

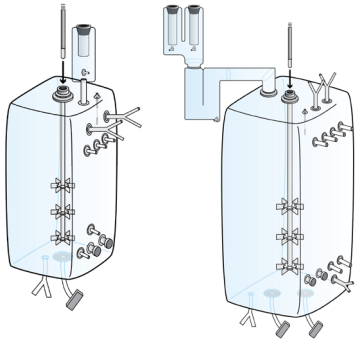

Figure 2. 30 L and 300 L S.U.F. bioprocess containers (BPCs) with condenser and foam sensor.

The agitator can sustain an agitation rate of 375–600 rpm and delivers 11 hp/1,000 gal (2.27 W/L) of mixing power, offering capacity beyond systems that use a magnetically coupled impeller. In addition, the modular design of the HyPerforma S.U.F. impeller drive train and tank baffles can be customized to meet specific culture or facility needs. Available in 30 L and 300 L sizes, the HyPerforma S.U.F. offers superior mass transfer performance and meets the process demands of dense and rapidly growing microbial cultures.

ThermoFisher SCIENTIFIC

A special high-flow version of the drilled hole sparge is provided as an integral component of the single-use fermentor. To better handle the high sparge rates of microbial systems, the S.U.F. condenser system was designed for airflow well beyond 2 vessel volumes/minute (vvm). It serves to both protect the filters from fouling and significantly reduce liquid evaporation loss during the process. All polymer components of the condenser system are fully integrated as single-use contact materials. Extensive application-based stress tests have demonstrated that S.U.F. high-flow filter options can sustain moist *E. coli* culture air at 2 vvm for continuous durations exceeding two weeks.

The gamma-irradiated S.U.F. eliminates the need for cleaning. Common cleaning procedures can take 1–2 days depending on stainless steel CIP treatments and sterilization procedures. The S.U.F. can be set up, media can be hydrated and sterile filtered, and probes can be calibrated in about 2 hours. The time, labor, and cleanup savings are substantial, and the S.U.F. also offers the benefit of eliminating cross-contamination risk. As proven with other single-use technologies, the S.U.F. offers the benefits of reduced initial capital investment, greatly simplified facility design, and much faster installation and process qualification.

The S.U.F. system also provides single-use options preconfigured into the single-use containers that facilitate process control monitoring of head-space gas pressure, foam generation, pH, dissolved oxygen (DO), and temperature. The S.U.F. vessel is designed to be retrofitted and integrated into existing fermentation controllers. End users should take note of options for measuring process liquid volume via load cells. In addition, pH, DO, or any other conventional 12 mm internal diameter (ID) SIP sensors can be safely integrated via the proven Thermo Scientific™ Single-use Bioreactor (S.U.B.) aseptic-type probe connector kits. The S.U.F. provides simple scale-up and robust transfer of microbial expression production procedures; even unique processes used in stainless steel CIP/SIP fermentors can be readily adapted to single-use technologies. In this application note, we compare the HyPerforma 30 L and 300 L S.U.F. to a traditional stainless steel fermentor for the production of an 80 kDa protein in *E. coli*.

Materials and methods

For *E. coli* testing, K12 medium was used with an exponential feed solution containing 75% glucose and 2.5% $MgSO_4$. K12 medium consisted of yeast extract (5 g/L), dextrose (25 g/L), KH_2PO_4 (2 g/L), K_2HPO_4 (3.13 g/L), $(NH_4)_2SO_4$ (5 g/L), $MgSO_4$ (1.22 g/L), thiamine (0.25 g/L), K12 trace element solution (1 mL/L), and kanamycin sulfate (0.025 g/L). K12 trace element solution was composed of (final concentrations given): $FeCl_3 \cdot 6H_2O$ (6.42 mg/L), NaCl (5 mg/L), $MnCl_2 \cdot 4H_2O$ (4 mg/L), $ZnSO_4 \cdot 7H_2O$ (1 mg/L), $CuSO_2 \cdot 5H_2O$ (0.4 mg/L), $Na_2MoO_4 \cdot 2H_2O$ (0.5 mg/L), H_3BO_3 (0.5 mg/L).

For the traditional stainless steel fermentor, 50 L of K12 medium was hydrated in the vessel and sterilized at 121°C for 20 min. Dextrose and $MgSO_4$ were sterilized in an autoclave and added to the medium after vessel sterilization. Trace elements were sterile filtered and added aseptically to sterile medium.

The K12 medium for the S.U.F. was hydrated in 25 L or 250 L batches with a Thermo Scientific™ HyPerforma™ Single-Use Mixer DS 300. Hydrated medium was filtered through a 0.45 µm filter followed by a sterile 2" 0.2 µm filter for the 30 L S.U.F. or a sterile 5" 0.2 µm filter for the 300 L S.U.F. A sterile media filter was connected via tube-welded C-Flex™ tubing (aseptic technique using quick connects is an option).

E. coli exponential fed-batch cultures

Exponential fed-batch cultures of *E. coli* strain BL21(DE3) expressing an 80 kDa protein were established with 30 L and 300 L working volumes in a HyPerforma S.U.F. (42 L and 420 L total volume) and in a traditional CIP/SIP fermentor with a 100 L working volume (New Brunswick BioFlo™ 610, 125 L total volume). The critical process parameters of the S.U.F. including pH, DO, temperature, liquid mass, foam level, and overlay pressure were controlled using Applikon™ S.U.F. bioprocess controllers configured with two 1 vvm mass flow controllers. The 316 stainless steel 100 L vessel has aspect ratios, impeller type, and impeller spacing similar to the S.U.F.

The *E. coli* seed cultures were prepared the day before protein production culture. High-density seed medium was filter sterilized, and 50 µg/mL of sterile kanamycin stock was added prior to inoculation. High-density seed medium was inoculated at 0.03% with freezer stock 12–16 hours prior to fermentor inoculation.

Seed volume was 1% of initial production volume. The cultures were grown at 37°C and 250 rpm overnight. Optical density (OD) at 600 nm was recorded, and seed culture was transferred aseptically through quick connects to 25 L, 250 L, or 50 L cultures in their respective vessels (30 L S.U.F., 300 L S.U.F., or 100 L traditional fermentor).

E. coli cultures were grown to optimize protein production. The pH was controlled at 7 ± 0.1 with 28% NH_4OH and 5 M H_2SO_4. Dissolved oxygen was set to 40% and controlled via cascade settings, with 400 stirrer rpm increased first, then air, followed by oxygen. The temperature was controlled at 37°C until the OD_{600} 300 reached 30 ± 2, at which point the temperature was set to 30°C. When the OD_{600} reached 40, IPTG was added to induce protein expression. Dry cell weight (DCW) samples were taken periodically for analysis.

Glucose was maintained at 20 ± 10 g/L until an OD_{600} of 130 ± 10 was achieved. Appropriate PharMed™ tubing for pump and feed rate was built into the feed-line set prior to irradiation and was connected to a feed bag of sterile glucose, aseptically, prior to initiating the feed program. Exponential feed program or manual set points were ramped up to maintain the optimal 20 ± 10 g/L concentration.

After the maximum OD was reached, the temperature was set to 10°C. Samples of all cultures were taken at 500 various time points. Part or all of culture pellets were collected, and protein was extracted and analyzed for protein production scale-up. Cells were collected for protein extraction via centrifugation at 21,000 rpm (CEPA Z41 high-speed centrifuge, 3" bowl ID). The protein was further processed for use as prototype products. At the completion of experiments, the single-use seed flasks were sterilized in a small autoclave prior to disposal.

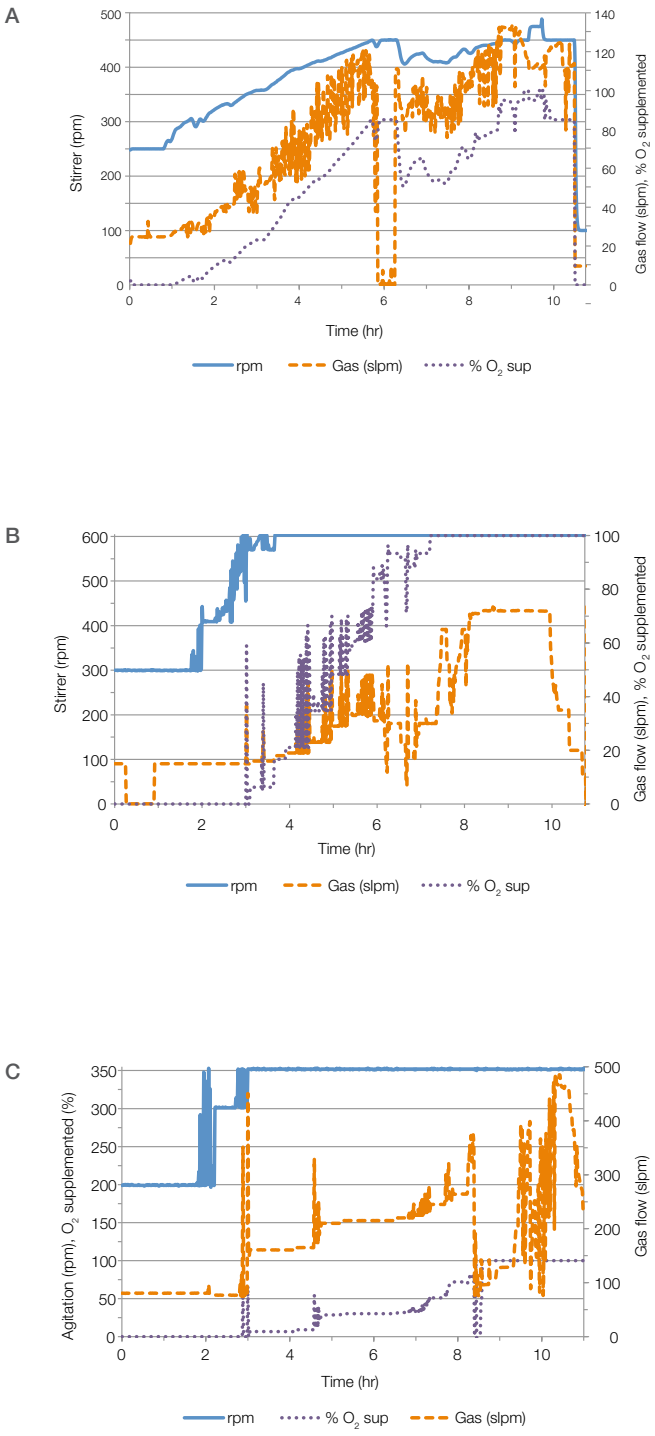

Figure 3. Exponential fed-batch *E. coli* cultures for 80 kDa protein production. Plots depict agitation, gas flow (standard liter per minute, slpm), and percent oxygen supplemented into gas flow. **(A)** Plot of 100 L traditional SIP fermentor culture. Initial volume was 50 L and final volume of the culture was approximately 75 L. **(B)** Plot of 30 L S.U.F. culture; final culture volume was approximately 30 L. **(C)** Plot of 300 L S.U.F. culture; final culture volume was approximately 300 L.

Results

Air flow rates in the traditional 100 L SIP fermentor reached above two times the liquid volume during various points of the exponential fed-batch *E. coli* culture. The culture OD at 600 nm reached 90 ± 10 by 10.5 hours. Gas flow rates for the traditional SIP fermentor were similar to those used in the S.U.F (Figure 3). Two experiments each were performed for the 30 L S.U.F. and the 300 L S.U.F. The first 30 L S.U.F. culture reached an OD_{600} of 140 with a final DCW of 47 g/L by 10 hours. The second 30 L S.U.F. culture reached an OD_{600} of 121 with a DCW of 45 g/L by 14 hours. Air flow rates of the 30 L S.U.F. culture reached above 2 vvm with the first culture and 1.7 vvm with the second culture.

The first 300 L culture reached an OD_{600} of 130 with a DCW of 39 g/L in 14 hours. The second 300 L culture reached an OD_{600} of 140 with a DCW of 36 g/L in 15 hours. From every 9.46 L centrifuged, about 1.35 kg of wet cell pellet was collected. The total wet cell mass was about 43 kg per 300 L culture. Air flow rates of the 300 L culture reached 1.6 vvm with the first culture and 1.4 vvm with the second culture.

The OD_{600} in S.U.F. experiments averaged 136 and DCW averaged 42 g/L (n = 4). As noted, the growth rates were similar to that of stainless steel SIP fermentors, though the SIP fermentor feed ended at an OD_{600} of 91 around 11 hours (Figure 4). The protein yields were similar in all runs (Figure 5).

Discussion

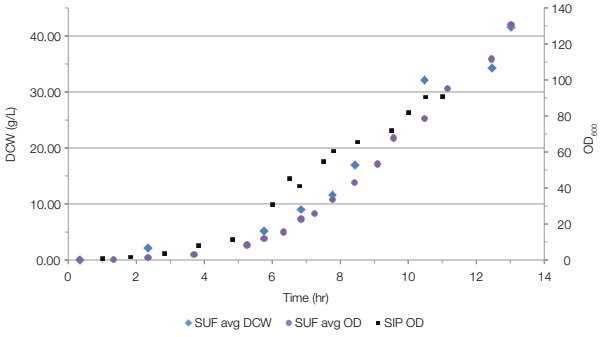

Figure 4. Culture growth rates. Graph depicts OD_{600} and DCW over time for *E. coli* cultures (n = 4) during 80 kDa protein expression in K12 medium with 2% glucose maintained through an exponential feed. S.U.F. data were taken from an average of two 30 L cultures and two 300 L cultures.

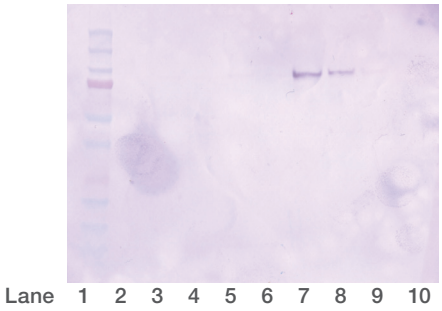

Figure 5. Western blot of expressed 80 kDa protein using anti-6xHis antibody. Lanes 2–6 are before induction, and lanes 7 and 8 are postinduction. Lane 1 is a protein ladder and lane 10 is empty. Similar gel images and protein yield were seen in SIP and S.U.F. cultures.

On average (n = 4), *E. coli* cultures at an OD_{600} of 136 with a DCW of 42 g/L were achieved in the S.U.F by 13 hours of culture. Equivalent expression of the 80 kDa protein was observed when compared to the traditional 100 L SIP vessel. The set points of the cultures were maintained throughout the culture. Pressure was maintained below the BPC operating optimal maximum of 0.5 psi (35 mbar). Agitation range and gas flow rates were sufficient at producing DO levels to maintain exponential growth and achieve the desired density and expression. Temperature control of the vessel met expectations. A small auxiliary heat exchanger was added between the temperature control unit (TCU) and vessel, which allowed a small flow of cold house water to rapidly cool the 300 L vessel prior to induction during high-density (OD_{600} of 30) exponential growth, when process was shifted from 37°C to 30°C.

The single-use condenser system prevented condensate from fouling the exhaust filter in these and previous cultures. Foam was monitored by the single-use foam sensor in these tests, allowing each run to be completed using only a single filter. TCUs for condensers were adequate for these cultures.

thermo scientific

Conclusions

The HyPerforma S.U.F. is revolutionary in being the first truly scalable fermentor capable of delivering production-level working volumes up to 300 L. Because each S.U.F. system offers a compact footprint and can be set up in just a few hours, now even industrial-scale output can be achieved using 3 or 4 of the 300 L S.U.F. vessels operated as a parallel batch (yielding 1,000–1,200 L of harvest). This technology allows cultures to be quickly scaled up with less effort, lower contamination risk, and more process flexibility.

These experiments confirm that very common technical transfer operations and process optimization methods are robust across working volumes (30 L and 300 L) of both systems and can also be directly applied when legacy processes are transferred to single-use technologies from conventional fermentation vessels. The critical performance parameters of heat transfer, liquid mixing, two-phase mass transfer, and process monitoring have been met in order to ensure that minimal effort is required to convert an existing process to a single-use production platform. The high performance demonstrated with this S.U.F. technology creates a new benchmark for features that should be important when choosing a system for microbial fermentation applications.

Find out more at **thermofisher.com/sut**

ThermoFisher SCIENTIFIC